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Change in the binding of hydrogen ions and magnesium ions in the hydrolysis of ATP

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Abstract

The binding of hydrogen ions and magnesium ions by a biochemical reactant, like ATP, can be calculated by writing the binding polynomial (partition function) Q and taking the partial derivatives of $\log Q$ with respect to pH and pMg by use of a mathematical program in a personal computer. The change in binding of hydrogen ions and magnesium ions in a biochemical reaction, like the hydrolysis of ATP, can be calculated by taking the partial derivatives of $\log(K'/K)$, where K' is the apparent equilibrium constant and K is the equilibrium constant for a reference chemical reaction. These calculations can be checked by using the computer to calculate the mixed partial derivatives, which must be equal. The effects of pH, pMg, and ionic strength on the changes in binding in the hydrolysis of ATP are calculated. At 298.15 K, pH 7, pMg 3, and 0.25 M ionic strength, the hydrolysis of ATP to ADP and inorganic phosphate liberates 0.62 mol of hydrogen ions and 0.45 mol of magnesium ions into the medium. © 1998 Elsevier Science B.V.

Keywords: Partition function; Binding of hydrogen ions; Binding of magnesium ions; Changes of binding in a reaction; Linkage

1. Introduction

The concept of the binding polynomial Q was developed in the study of the binding of ligands by proteins (Klotz [1], Wyman [2], Wyman and Gill [3], Di Cera [4], Klotz [5], Schellman [6]). The binding polynomial is really a partition function and has other uses beyond the calculation of amounts bound, but here, the binding of ligands is emphasized. The partition function is useful because the average number $\bar{N}_{\mathrm{L}}$ of ligand molecules bound per macromolecule is equal to the derivative of the natural logarithm of the partition function Q with respect to the natural logarithm of the free concentration of the ligand at constant temperature and pressure:

$$\bar{N}_L = \left(\frac{\mathrm{d}\ln Q}{\mathrm{d}\ln[L]}\right)_{\mathrm{T,P}} = \frac{[L]}{Q}\left(\frac{\mathrm{d}Q}{\mathrm{d}[L]}\right)_{\mathrm{T,P}} \tag{1}$$

When the binding of two ligands is competitive, the average numbers of molecules of the two ligands bound per macromolecule are given by

$$\bar{N}_{L_1} = \left(\frac{\partial \ln Q}{\partial \ln[L_1]}\right)_{\mathrm{T,P},[L_2]} \tag{2}$$

$$\bar{N}_{L_2} = \left(\frac{\partial \ln Q}{\partial \ln[L_2]}\right)_{\mathrm{T,P},[L_1]} \tag{3}$$

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Since the partition function Q is an exact differential, the mixed partial derivatives are equal:

$$\left(\frac{\partial^2 \ln Q}{\partial \ln[L_1]\partial \ln[L_2]}\right)_{T,P} = \left(\frac{\partial^2 \ln Q}{\partial \ln[L_2]\partial \ln[L_1]}\right)_{T,P} \quad (4)$$

Substituting Eqs. (2) and (3) yields

$$\left(\frac{\partial \overline{N}_{L_1}}{\partial \ln[L_2]}\right)_{T,P} = \left(\frac{\partial \overline{N}_{L_2}}{\partial \ln[L_1]}\right)_{T,P} \quad (5)$$

Thus, the effect of changing the concentration of L_1 on the binding of L_2 is the same as the effect of changing the concentration of L_2 on the binding of L_1. This shows the linkage between the bindings of the two ligands.

This terminology has not been used so much in studying the binding of ligands by small molecules, but it is equally applicable. The example discussed here is the binding of H^+ and Mg^{2+} by adenosine triphosphate, but the principal objective of this article is to apply these methods to the calculation of the change in binding of H^+ and metal ions in biochemical reactions, as illustrated by the hydrolysis of ATP to ADP and inorganic phosphate.

The change in binding of hydrogen ions and magnesium ions in biochemical reactions was discussed some time ago (Alberty [7]), but without using Eqs. (2) and (3). More recently, the fundamental equation of thermodynamics for the transformed Gibbs energy has been used [8] to show that the change in binding of hydrogen ions $\Delta_r N(H^+)$ in a biochemical reaction is given by

$$\Delta_r N(H^+) = -\left(\frac{\partial \log K'}{\partial pH}\right)_{T,P,pMg} \quad (6)$$

where K' is the apparent equilibrium constant of the biochemical reaction. The corresponding equation for the change in binding of magnesium ions is

$$\Delta_r N(Mg^{2+}) = -\left(\frac{\partial \log K'}{\partial pMg}\right)_{T,P,pH} \quad (7)$$

This nomenclature is consistent with that for the calculation of other thermodynamic properties, like $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ for biochemical reactions, where the primes indicate that the pH and pMg are specified. The reason the above equations are important from a computational standpoint is that when the binding polynomial is large or K' involves a large number of terms, a personal computer can be used to take a partial derivative (for example, *Mathematica* [9]) so that Eqs. (2) and (3) can be used to plot the binding of H^+ and a metal ion, and Eqs. (6) and (7) can be used to calculate the change in binding in a biochemical reaction without the necessity of doing mathematical operations on the partition function by hand.

Calculations of changes in binding of H^+ and Mg^{2+} in the hydrolysis of ATP were calculated earlier [6] using

$$\Delta_r N(H^+) = \Sigma \upsilon_i \overline{N}_H(i) \quad (8)$$

and

$$\Delta_r N(Mg^{2+}) = \Sigma \nu_i \overline{N}_{Mg}(i) \quad (9)$$

where the average number $\overline{N}_H(i)$ of hydrogen ions bound by reactant i is given by $\Sigma f_j N_H(j)$, where f_j is the fraction of reactant i in species j and $N_H(j)$ is the number of hydrogen ions in species j. The stoichiometric number of reactant i in the biochemical equation is represented by ν_i. For a reaction like the hydrolysis of ATP, a lot of work is involved in calculating $\Delta_r N(H^+)$ and $\Delta_r N(Mg^{2+})$ by hand because ATP in the presence of magnesium ions involves six species, ADP involves five species, and P_i involves three species.

2. Calculation of average numbers of hydrogen ions and magnesium ions bound by ATP

Because of the importance of ATP in energy transfer, a great deal of research has been done on the acid dissociation constants and dissociation constants of complex ions of ATP, ADP, AMP, and P_i. Goldberg and Tewari [10] and Lawson et al. [11] have critically evaluated these data and recommended the values given in Table 1 at zero ionic strength.

The values of equilibrium constants and apparent equilibrium constants throughout this paper are taken as dimensionless quantities so that they are suitable arguments for logarithms. Concentrations are in mol l^{-1} but are also treated as dimensionless. The values

Table 1
Dissociation constants for species involved in the hydrolysis of ATP to ADP and inorganic phosphate at 298.15 K and 1 Bar

Chemical reaction	$K(I=0)$	$K(I=0.1\text{ M})$	$K(I=0.25\text{ M})$
$HATP^{3-} = H^{+} + ATP^{4-}$	2.512×10^{-8}	1.811×10^{-7}	3.426×10^{-7}
$H_2ATP^{2-} = H^{+} + HATP^{3-}$	2.089×10^{-5}	9.190×10^{-5}	1.483×10^{-4}
$MgATP^{2-} = Mg^{2+} + ATP^{4-}$	6.607×10^{-7}	3.433×10^{-5}	1.229×10^{-4}
$MgHATP^{-} = Mg^{2+} + HATP^{3-}$	2.344×10^{-4}	4.536×10^{-3}	1.181×10^{-2}
$Mg_2ATP = Mg^{2+} + MgATP^{2-}$	2.042×10^{-3}	1.472×10^{-2}	2.785×10^{-2}
$HADP^{2-} = H^{+} + ADP^{3-}$	6.607×10^{-8}	2.907×10^{-7}	4.689×10^{-7}
$H_2ADP^{-} = H^{+} + ADP^{2-}$	4.365×10^{-5}	1.1172×10^{-4}	1.612×10^{-4}
$MgADP^{-} = Mg^{2+} + ADP^{3-}$	2.239×10^{-5}	4.333×10^{-4}	1.128×10^{-3}
$MgHADP = Mg^{2+} + HADP^{2-}$	3.162×10^{-3}	2.279×10^{-2}	4.313×10^{-3}
$H_2PO_4^{-} = H^{+} + HPO_4^{2-}$	6.026×10^{-8}	1.618×10^{-7}	2.225×10^{-7}
$MgHPO_4 = Mg^{2+} + HPO_4^{2-}$	1.950×10^{-3}	1.406×10^{-2}	2.660×10^{-2}

of equilibrium constants at specified ionic strengths I have been calculated using

$$\log K(I) = \log K(I=0) + \left(0.51065\, I^{1/2} \Sigma \nu_i z_i^2\right) / \left(1 + B I^{1/2}\right) \qquad (10)$$

where z_i is the charge number of species i, ν_i is its stoichiometric number, and $B = 1.6$ $l^{1/2}$ $mol^{-1/2}$ (Clarke and Glew [12]). Alberty and Goldberg [13] have used the values in Table 1 at $I = 0.25$ M in the calculation of standard transformed Gibbs energies and standard transformed enthalpies of formation for the ATP series and several related reactants at 298.15 K, pH 7, pMg 3, and 0.25 M ionic strength. Eqs. (8) and (9) and the values in Table 1 can be used to calculate $\bar{N}_H(\text{ATP})$, $\bar{N}_H(\text{ADP})$, $\bar{N}_H(\text{P}_i)$, $\bar{N}_{Mg}(\text{ATP})$, $\bar{N}_{Mg}(\text{ADP})$, and $\bar{N}_{Mg}(\text{P}_i)$ using $\Sigma f_j N_H(\text{j})$ and $\Sigma f_j N_{Mg}(\text{j})$, but this is avoided in this paper by writing out the equations for the partition functions for these three reactants and using mathematical programs to calculate the partial derivatives in Eqs. (2)–(7).

The binding polynomial for ATP can be obtained by using the expression for [ATP] as the sum of species concentrations:

$$[\text{ATP}] = [\text{ATP}^{4-}] + [\text{HATP}^{3-}] + [\text{H}_2\text{ATP}^{2-}] + [\text{MgATP}^{2-}] + [\text{MgHATP}^{-}] + [\text{Mg}_2\text{ATP}] \qquad (11)$$

By use of the equilibrium constant expressions for the dissociations in Table 1, this equation can be written in the form

$$\frac{[\text{ATP}]}{[\text{ATP}^{4-}]} = 1 + \frac{[\text{H}^+]}{K_{1\text{ATP}}} + \frac{[\text{H}^+]^2}{K_{1\text{ATP}}K_{2\text{ATP}}} + \frac{[\text{Mg}^{2+}]}{K_{3\text{ATP}}} + \frac{[\text{Mg}^{2+}][\text{H}^+]}{K_{1\text{ATP}}K_{4\text{ATP}}} + \frac{[\text{Mg}^{2+}]^2}{K_{3\text{ATP}}K_{5\text{ATP}}}. \qquad (12)$$

The polynomial on the right is the binding polynomial, and it can be written as

$$Q(\text{ATP}) = 1 + \frac{10^{-\text{pH}}}{K_{1\text{ATP}}} + \frac{10^{-2\,\text{pH}}}{K_{1\text{ATP}}K_{2\text{ATP}}} + \frac{10^{-\text{pMg}}}{K_{3\text{ATP}}} + \frac{10^{-\text{pH}-\text{pMg}}}{K_{1\text{ATP}}K_{4\text{ATP}}} + \frac{10^{-2\,\text{pMg}}}{K_{3\text{ATP}}K_{5\text{ATP}}} \qquad (13)$$

Since it is convenient to write this polynomial for ATP in terms of $\text{pH} = -\log[\text{H}^+]$ and $\text{pMg} = -\log[\text{Mg}^{2+}]$, it is useful to write Eqs. (2) and (3) in the forms

$$\bar{N}_H(\text{ATP}) = -\left(\frac{\partial \log Q(\text{ATP})}{\partial \text{pH}}\right)_{T,P,\text{pMg}} \qquad (14)$$

$$\bar{N}_{Mg}(\text{ATP}) = -\left(\frac{\partial \log Q(\text{ATP})}{\partial \text{pMg}}\right)_{T,P,\text{pH}} \qquad (15)$$

Since *Mathematica* can be used to calculate partial

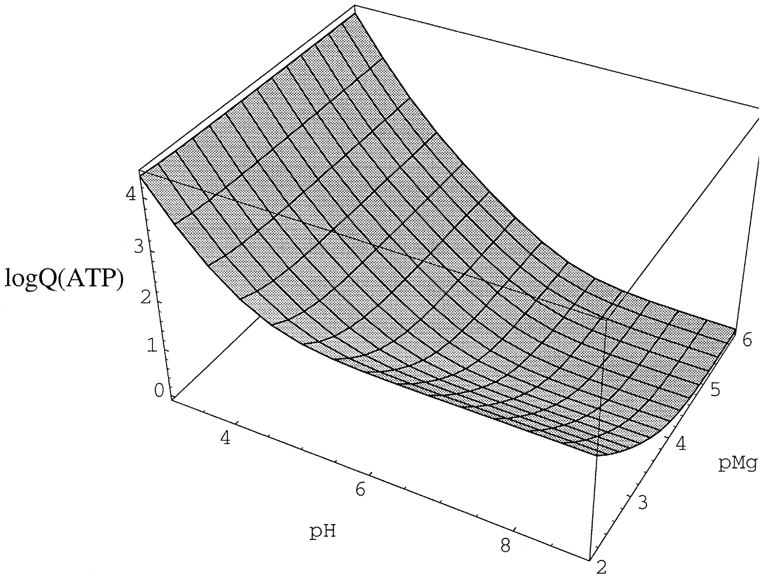

Fig. 1. Plot of log Q(ATP) at 298.15 K and 0.25 M ionic strength vs. pH and pMg. The slope of this plot in the pH direction is -1 times the binding of hydrogen ions by ATP, and the slope in the pMg direction is -1 times the binding of magnesium ions.

derivatives, Eqs. (14) and (15) can be used to calculate $\bar{N}_H$(ATP) and $\bar{N}_{Mg}$(ATP) at the desired T, P, pH, pMg, and I or make three-dimensional plots.

It is of interest to plot log$Q$(ATP) vs. pH and pMg because this plot contains all of the information about $\bar{N}_H$(ATP) and $\bar{N}_{Mg}$(ATP). The plot of

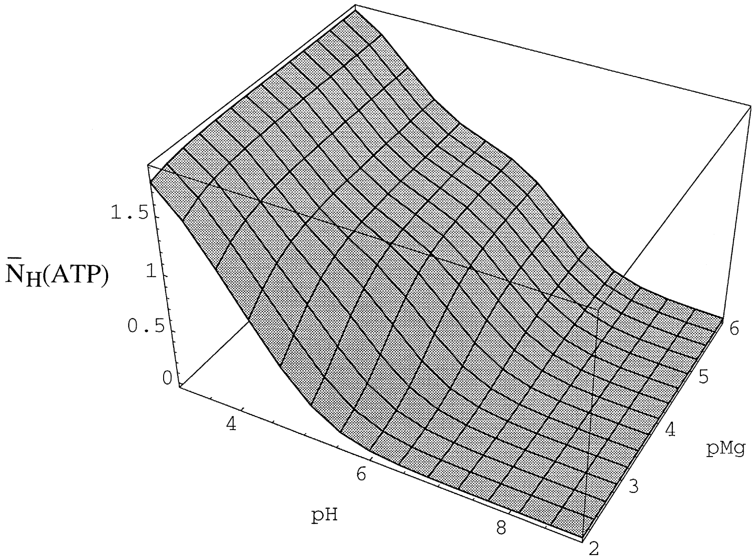

Fig. 2. Plot of the average number of hydrogen ions bound by ATP at 298.15 K and 0.25 M ionic strength calculated using Eq. (14).

$\log Q(\text{ATP})$ is given in Fig. 1. At any specified pH and pMg, the slope of this plot in the pH direction is equal to $-\bar{N}_{\text{H}}(\text{ATP})$ and the slope of the plot in the pMg direction is $-\bar{N}_{\text{Mg}}(\text{ATP})$. Since $Q(\text{ATP})$ is an exact differential, the two mixed partial derivatives are equal.

$$\left(\frac{\partial^2 \log Q(\text{ATP})}{\partial \text{pMg}\,\partial \text{pH}}\right)_{\text{T,P}} = \left(\frac{\partial^2 \log Q(\text{ATP})}{\partial \text{pH}\,\partial \text{pMg}}\right)_{\text{T,P}} \qquad (16)$$

Thus,

$$\left(\frac{\partial \bar{N}_H(\,ATP)}{\partial pMg}\right)_{\text{T,P}} = \left(\frac{\partial \bar{N}_{Mg}(\,ATP)}{\partial pH}\right)_{\text{T,P}} \qquad (17)$$

Eq. (17), which was given earlier for inorganic phosphate [14], expresses the linkage between the binding of H^+ and Mg^{2+}. It also provides a check on calculations, or experiments.

Fig. 2 gives the average number of hydrogen ions bound by ATP calculated using Eq. (14). The right back face gives $\bar{N}_{\text{H}}$ in the absence of Mg^{2+}, and the left front face gives $\bar{N}_{\text{H}}$ at pMg 2. These two faces are really acid titration curves. The number $\bar{N}_{\text{H}}$ is relative to ATP^{4-}, for which $\bar{N}_{\text{H}} = 0$. Note that the apparent pK of ATP in the neighborhood of pH 7 is reduced by Mg^{2+}. This reduction in apparent pK is the basis for the experimental method for determining the dissociation constants of the complex ions of ATP containing Mg^{2+}.

The average number of magnesium ions bound by ATP is calculated using Eq. (15) and is given by Fig. 3. At low pH, Mg^{2+} is not bound significantly because the binding by HATP^{3-} is weaker than the binding by ATP^{4-}, and there is no evidence of binding by $\text{H}_2\text{ATP}^{2-}$. The p$K$ for the binding of the second Mg^{2+} by ATP^{4-} is about 1.5, and therefore binding does not get much above 1.0 at pMg 2.

Since $Q(\text{ATP})$ is an exact differential, one check of the validity of these calculations is that the mixed partial derivatives are equal as required by Eq. (17). The slope of Fig. 3 in the pH direction at each pH, pMg must be equal to the slope of Fig. 2 in the pMg direction at that pH, pMg. Fig. 4 gives the partial derivative of $\bar{N}_{\text{Mg}}(\text{ATP})$ with respect to pH. An identical plot is obtained by taking the derivative of $\bar{N}_{\text{H}}(\text{ATP})$ with respect to pMg. Thus, the plots in Figs. 2 and 3 are related in an interesting way: at a given pH and pMg, the slope of Fig. 2 in the pMg

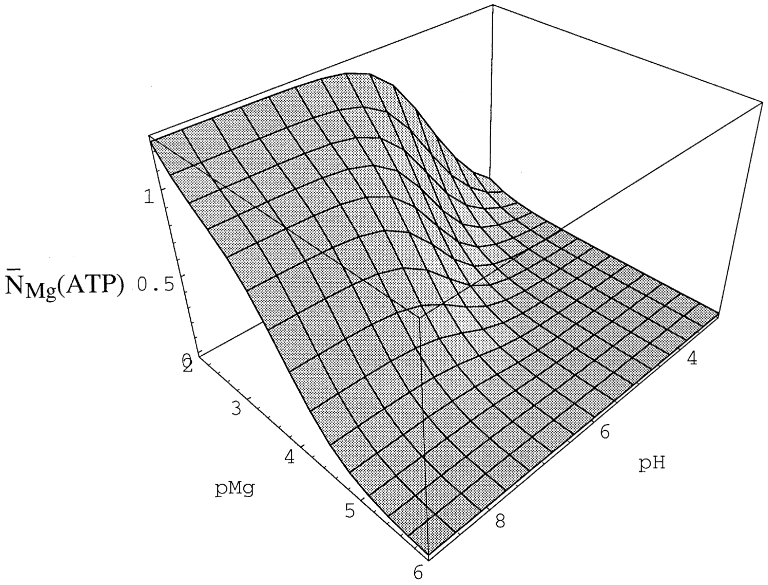

Fig. 3. Plot of the average number of magnesium ions bound by ATP at 298.15 K and 0.25 M ionic strength calculated using Eq. (15).

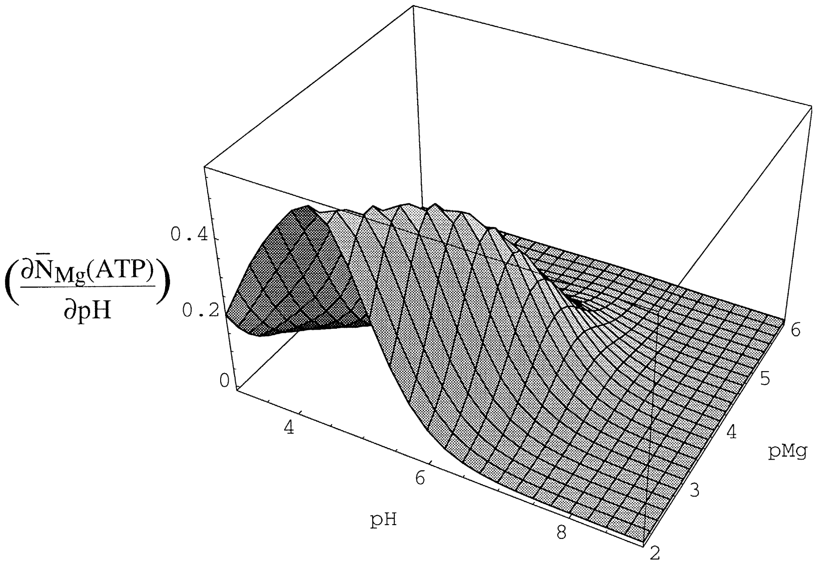

Fig. 4. Plot of the partial derivative of the average number of magnesium ions bound by ATP with respect to pH at 298.15 K and 0.25 M ionic strength. The same plot is obtained with the partial derivative of the average number of hydrogen ions bound with respect to the pMg, in agreement with Eq. (5).

direction is equal to the slope of Fig. 3 in the pH direction at the corresponding pH and pMg.

3. Calculation of change in the number of bound hydrogen ions and bound magnesium ions in the hydrolysis of ATP

For the hydrolysis of ATP to ADP and P_i at specified pH and pMg,

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

the apparent equilibrium constant K' is given by

$$K' = \frac{[ADP][P_i]}{[ATP]} = \frac{K}{[H^+]}\frac{Q(ADP)Q(P_i)}{Q(ATP)} \tag{19}$$

where K is the equilibrium constant for the reference chemical reaction

$$ATP^{4-} + H_2O = ADP^{3-} + HPO_4^{2-} + H^+ \quad K = \frac{[ADP^{3-}][HPO_4^{2-}][H^+]}{[ATP^{4-}]} \tag{20}$$

This equation shows the reference states for the reactants; binding of H^+ by ATP is with respect to ATP^{4-}, binding of H^+ by ADP is with respect to ADP^{3-}, and binding of H^+ by P_i is with respect to PO_4^{3-}.

Eq. (19) can be written in logarithmic form as

$$\log\left(\frac{K'}{K}\right) = \log Q(ADP) + \log Q(P_i) - \log Q(ATP) - \log[H^+] \tag{21}$$

This is the function that determines the change in binding of H^+ and the change in binding of Mg^{2+} in the hydrolysis of ATP. As indicated by Eq. (6) the change in binding of H^+ in the biochemical reaction is obtained by taking the negative partial derivative of this function with respect to pH at constant pMg:

$$\Delta_r N(H^+) = -\frac{\partial \log(K'/K)}{\partial pH} = -\frac{\partial \log Q(ADP)}{\partial pH} - \frac{\partial \log Q(P_i)}{\partial pH} + \frac{\partial \log Q(ADP)}{\partial pH} - 1 \tag{22}$$

The change in binding of Mg^{2+} is obtained by

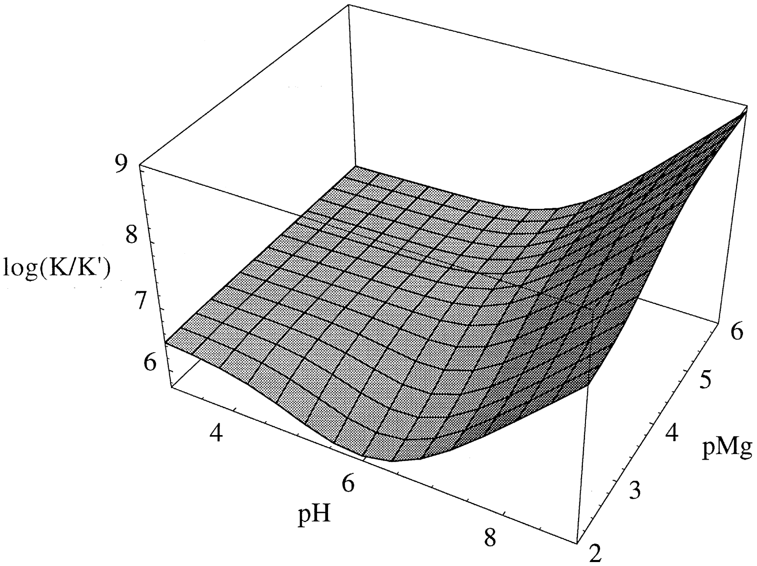

Fig. 5. Plot of $\log(K'/K)$ for ATP hydrolysis at 298.15 K and 0.25 M ionic strength vs. pH and pMg. The slope of this plot in the pH direction gives -1 times the change in binding of hydrogen ions in the reaction, and the slope in the pMg direction gives -1 times the change in binding of magnesium ions.

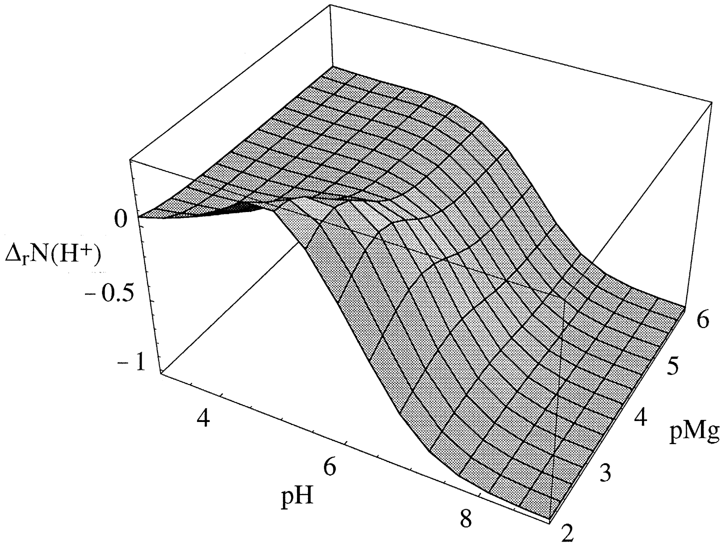

Fig. 6. Plot of the change in the binding of hydrogen ions in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength. The right back plane shows the change in the binding of hydrogen ions in the absence of magnesium ions, and the left front plane shows the change in binding of hydrogen ions at pMg 2.

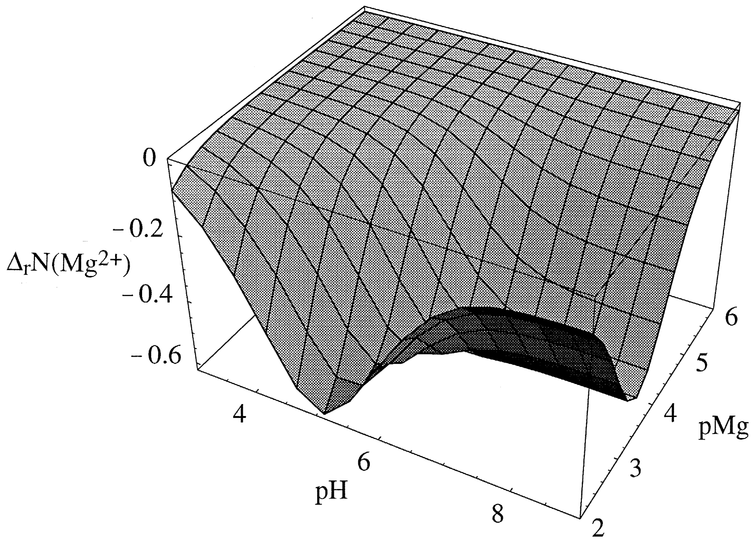

Fig. 7. Plot of the change in the binding of magnesium ions in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength. The left back plane shows the change in the binding of magnesium ions at pH 3, and the right front plane shows the change in binding of magnesium ions at pH 9.

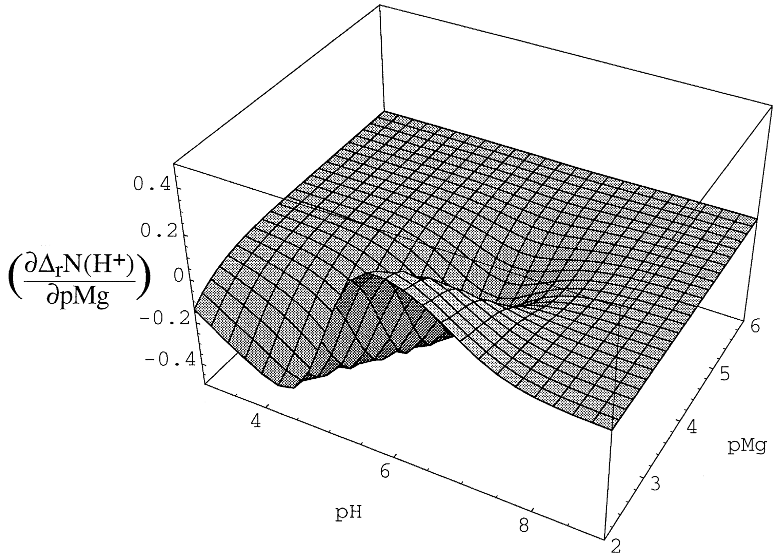

Fig. 8. Plot of the partial derivative of the change in binding of hydrogen ions in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength with respect to pMg. An identical plot is obtained by taking the partial derivative of the change in binding of magnesium ions with respect to pH, as expected from Eq. (25).

Table 2
$\Delta_r N(H^+)$ in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength

pH	pMg				
	2	3	4	5	6
3	0.06	0	0	−0.01	−0.01
4	0.20	0.01	−0.02	−0.02	−0.02
5	0.40	0.13	−0.02	−0.04	−0.04
6	−0.07	0.10	−0.14	−0.23	−0.25
7	−0.73	−0.62	−0.67	−0.73	−0.74
8	−0.97	−0.95	−0.95	−0.96	−0.96
9	−1.00	−0.99	−1.00	−1.00	−1.00

taking the negative partial derivative of $\log(K'/K)$ with respect to pMg at constant pH:

$$\Delta_r N(\mathrm{Mg}^{2+}) = -\frac{\partial\log(K'/K)}{\partial\mathrm{pMg}} = -\frac{\partial\log\mathrm{P(ADP)}}{\partial\mathrm{pMg}} - \frac{\partial\log\mathrm{P(P_i)}}{\partial\mathrm{pMg}} + \frac{\partial\log\mathrm{P(ADP)}}{\partial\mathrm{pMg}} \tag{23}$$

Note that the mixed partial derivatives of $\log(K'/K)$ have to be equal because $\log(K'/K)$ is an exact differential:

$$\left(\frac{\partial\log(K'/K)}{\partial\mathrm{pMg}\,\partial\mathrm{pH}}\right)_{T,P} = \left(\frac{\partial\log(K'/K)}{\partial\mathrm{pH}\,\partial\mathrm{pMg}}\right)_{T,P}. \tag{24}$$

Eqs. (22) and (23) lead to

$$\left(\frac{\partial\Delta_r N(\mathrm{H}^+)}{\partial\mathrm{pMg}}\right)_{T,P} = \left(\frac{\partial\Delta_r N(\mathrm{Mg}^{2+})}{\partial\mathrm{pH}}\right)_{T,P} \tag{25}$$

which expresses the linkage between the changes in binding of H^+ and Mg^{2+}.

The dependence of $\log(K'/K)$ at 298.15 K and $I = 0.25$ M on pH and pMg is given by Fig. 5.

The slope in the pH direction gives $-\Delta_r N(H^+)$, and the slope in the pMg direction gives $-\Delta_r N(Mg^{2+})$. These slopes are shown in Figs. 6 and 7.

The fact that the slope of Fig. 6 is negative in the neighborhood of pH 7 means that ADP and P_i bind less H^+ than the ATP and H_2O they are formed from so that hydrogen ions are liberated into the solution when Eq. (18) occurs in the neighborhood of pH 7. This has to be taken into account in calorimetric experiments because the H^+ produced reacts with the buffer to produce or consume heat, according to the enthalpy change for the acid dissociation of the buffer acid (Alberty and Goldberg [15]). Note that although the change in binding of H^+ is negative in most of the neutral pH range, it is positive at high magnesium concentrations below pH 6.

Since $\Delta_r N(Mg^{2+})$ is negative in the neighborhood of pH 7, Mg^{2+} is liberated into the solution by Eq. (18). This is a result of the fact that the binding of Mg^{2+} to ATP is greater than the binding to ADP and P_i combined.

Again the mixed partial derivatives provide a test of these calculations. The plot in Fig. 8 is obtained by taking the derivative $\partial\Delta_r N(H^+)/\partial\mathrm{pMg}$ or $\partial\Delta_r N(Mg^{2+})/\partial\mathrm{pH}$, as required by Eq. (25). This is a test that is very difficult to make by hand because of the very large number of terms. The mixed partial derivatives identify the region of pH and pMg where changing the pH has the largest effect on $\Delta_r N(Mg^{2+})$ and changing pMg has the largest effect on $\Delta_r N(H^+)$.

4. Calculation of the effect of ionic strength on the change in the binding of hydrogen ions and magnesium ions in the hydrolysis of ATP

Table 2 gives the changes in the binding of H^+ in the hydrolysis of ATP to ADP and inorganic phosphate at seven values of the pH and five values of pMg calculated using Eq. (22).

Table 3 gives the changes in the binding of Mg^{2+} in the hydrolysis of ATP at seven values of the pH and five values of pMg using Eq. (23). To show the

Table 3
$\Delta_r N(Mg^{2+})$ in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength

pH	pMg				
	2	3	4	5	6
3	−0.07	−0.01	0	0	0
4	−0.31	−0.05	−0.01	0	0
5	−0.61	−0.20	−0.03	0	0
6	−0.40	−0.47	−0.15	−0.02	0
7	−0.16	−0.45	−0.32	−0.05	−0.01
8	−0.09	−0.42	−0.36	−0.06	−0.01
9	−0.08	−0.42	−0.37	−0.07	−0.01

Table 4
$\Delta_r N(H^+)$ in the hydrolysis of ATP at 298.15 K and 0.10 M ionic strength

pH	pMg				
	2	3	4	5	6
3	0.09	0	−0.02	−0.02	−0.02
4	0.29	0.01	−0.06	−0.06	−0.04
5	0.42	0.22	0	−0.04	−0.05
6	−0.08	0.20	0.01	−0.18	−0.20
7	−0.72	−0.56	−0.53	−0.67	−0.71
8	−0.96	−0.94	−0.93	−0.95	−0.96
9	−1.00	−0.99	−0.99	−0.99	−1.00

effect of ionic strength Tables 2 and 3 were recalculated at $I = 0.10$ M; the changes in binding at $I = 0.10$ M are shown in Tables 4 and 5.

The change in binding of H^+ with this change in ionic strength is as much as 0.15, and the change in binding of Mg^{2+} is as much as 0.19. At pH 7, $\Delta_r N(H^+)$ is negative at all pMg, but at pH 6 and lower pH, $\Delta_r N(H^+)$ is positive at low pMg. This happens because of the growing importance of the chemical reaction

$$H^+ + MgATP^{2-} + H_2O = HADP^{2-} + H_2PO_4^- + Mg^{2+} \qquad (26)$$

which reduces the concentration of hydrogen ions in the solution and increases the concentration of magnesium ions. At pH 4–5 and pMg 2, each H^+ produces about 1.5 Mg^{2+}.

Thus the ‘stoichiometry’ of H^+ and Mg^{2+} discussed here is different from the stoichiometry of ATP, ADP, and P_i, which is indpendent of T, P, pH, pMg, and ionic strength. The ‘stoichiometry’ of H^+ and Mg^{2+} is quite different; it is a function of all of these variables.

Table 5
$\Delta_r N(Mg^{2+})$ in the hydrolysis of ATP at 298.15 K and 0.10 M ionic strength

pH	pMg				
	2	3	4	5	6
3	−0.12	−0.01	0	0	0
4	−0.45	−0.09	−0.01	0	0
5	−0.69	−0.34	−0.05	−0.01	0
6	−0.44	−0.52	−0.27	−0.04	0
7	−0.15	−0.34	−0.51	−0.14	−0.02
8	−0.04	−0.28	−0.55	−0.19	−0.03
9	−0.03	−0.27	−0.56	−0.20	−0.03

5. Discussion

Biochemistry textbooks have many errors in the stoichiometry they indicate for H^+ in biochemical reactions catalyzed by single enzymes at pH 7. For example, many textbooks write $ATP + H_2O = ADP + P_i + H^+$, and include hydrogen ions in many other reactions at specified pH, including net reactions. This is incorrect stoichiometrically at pH 7 and does not follow the recommendations of the IUBMB-IUPAC Report [16,17] that when pH is specified, the biochemical equation should omit H^+ because it is not conserved. The IUBMB recommendations on biochemical nomenclature (Webb [18]) show H^+ in a few reactions, but the people responsible are in the process of removing them. This does not mean that the liberation of hydrogen ions in the hydrolysis of ATP is not important; it appears to be very important in the operation of nitrogenase at pH 7 because 10 mol of H^+ is consumed in the reduction of a mol of N_2 to ammonia [19] and enough ATP is hydrolyzed to provide 10 mol of H^+.

These discussions have been based on $\log(K'/K)$. Discussions of $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ are based on $\log K'$ [8].

In reactions like Eq. (18), there are also changes in the binding of water molecules. In principle, these changes can be studied by including terms for the binding of H_2O in the partition function, as discussed by Klotz [20] and Tanford [21]. In practice, there is a problem when the activity of water is changed by adding a large amount of another substance because the pH scale is only defined for dilute aqueous solutions.

The laborious calculations of $\Delta_r N(H^+)$ and $\Delta_r N(Mg^{2+})$ for a biochemical reaction using Eqs. (8) and (9) are avoided here by use of the partial derivative built-in function of *Mathematica*. The single program given in Appendix A can be used to calculate the effects of pH, pMg, and I on the binding of H^+ and Mg^{2+} by ATP, ADP, and P_i and the changes in binding in the hydrolysis of ATP to ADP and inorganic phosphate.

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Appendix A

```
plot3Dlogqatp[is_] := Module[{k0, sz2, ki, qatp, qadp, qpi, nHatp, nHadp, nHpi,
   nMgatp, nMgadp, nMgpi, nHMgatp, nHMgadp, nHmgpi, kprime, divpH, divpMg},
  k0 = {2.512 * 10 ^ -8, 2.089 * 10 ^ -5, 6.607 * 10 ^ -7, 2.344 * 10 ^ -4, 2.042 * 10 ^ -3,
    6.607 * 10 ^ -8, 4.365 * 10 ^ -5, 2.239 * 10 ^ -5, 3.162 * 10 ^ -3, 6.026 * 10 ^ -8, 1.950 * 10 ^ -3};
  sz2 = {8, 6, 16, 12, 8, 6, 4, 12, 8, 4, 8};
  ki = k0 * Exp[((Log[10] * .51065 * is ^ .5) / (1 + 1.6 * is ^ .5)) * sz2];
  qatp = 1 + (10 ^ -pH) / ki[[1]] + ((10 ^ -pH) ^ 2) / (ki[[1]] * ki[[2]]) + ((10 ^ -pMg) / ki[[3]]) +
    ((10 ^ -pH) * (10 ^ -pMg)) / (ki[[1]] * ki[[4]]) + ((10 ^ -pMg) ^ 2) / (ki[[3]] * ki[[5]]);
  qadp = 1 + (10 ^ -pH) / ki[[6]] + ((10 ^ -pH) ^ 2) / (ki[[6]] * ki[[7]]) +
    (10 ^ -pMg) / ki[[8]] + ((10 ^ -pH) * (10 ^ -pMg)) / (ki[[6]] * ki[[9]]);
  qpi = 1 + (10 ^ -pH) / ki[[10]] + ((10 ^ -pMg) / ki[[11]]);
  nHatp = (-1 / Log[10]) * D[Log[qatp], pH]; nHadp = (-1 / Log[10]) * D[Log[qadp], pH];
  nHpi = (-1 / Log[10]) * D[Log[qpi], pH];
  nMgatp = (-1 / Log[10]) * D[Log[qatp], pMg]; nMgadp = (-1 / Log[10]) * D[Log[qadp], pMg];
  nMgpi = (-1 / Log[10]) * D[Log[qpi], pMg];
  nHMgatp = D[nHatp, pMg];
  nHMgadp = D[nHadp, pMg];
  nHMgpi = D[nHpi, pMg];
  kprime = (qadp * qpi) / ((10 ^ -pH) * qatp);
  divpH = (-1 / Log[10]) * D[Log[kprime], pH];
  divpMg = (-1 / Log[10]) * D[Log[kprime], pMg];
  Plot3D[(1 / Log[10]) * Log[qatp], {pH, 3, 9},
   {pMg, 2, 6}, AxesLabel -> {"pH", "pMg", ""}, BoxRatios -> {1.5, 1.2, .9}]]
```

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