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Thermodynamics of the disproportionation of adenosine 5′-diphosphate to adenosine 5′-triphosphate and adenosine 5′-monophosphate

I. Equilibrium model

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The thermodynamic treatment of the disproportionation reaction of adenosine 5′-diphosphate to adenosine 5′-triphosphate and adenosine 5′-monophosphate is discussed in terms of an equilibrium model which includes the effects of the multiplicity of ionic and metal bound species and the presence of long range electrostatic and short range repulsive interactions. Calculated quantities include equilibrium constants, enthalpies, heat capacities, entropies, and the stoichiometry of the overall reaction. The matter of how these calculations can be made self-consistent with respect to both calculated values of the ionic strength and the molality of the free magnesium ion is discussed. The thermodynamic data involving proton and magnesium-ion binding data for the nucleotides involved in this reaction have been evaluated.

1. Introduction

A commonly occurring problem in the treatment of biochemical equilibria is the observed dependency of equilibrium constants, enthalpies of reaction, and the stoichiometry of the reaction itself upon variables such as pH, temperature, metal-ion concentration, and ionic strength. The underlying cause of these variations is the multiplicity of ionic and metal bound species of the molecules participating in the reaction, as well as the presence of both long range electrostatic and short range interactions between the various solute species. The consistent and systematic treatment of such data requires the use of a model which includes the combined effects of these variables and thus has the potential to predict the values of measurable quantities under experimental conditions which have not been the subject of investigation. Also, it is frequently desirable to adjust measured values to a common standard state both as a basis of comparison with results from other laboratories and as a prerequisite to performing thermochemical cycle calculations. In this paper a model is described which uses thermodynamic parameters for the various reactions occurring in solution together with an appropriate expression for the activity coefficients of the species in solution. The use of such a model has the potential to account for many of the afore mentioned interactions. Calculations of a similar nature have been performed by Alberty [1,2], Phillips et al. [3], Guynn et al. [4], and others on a variety of enzyme-catalyzed reactions. For the most part, however, these calculations have been performed at a constant ionic strength and did not introduce any systematic corrections for the activity coefficients of the species in solution. A rigorous treatment

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also requires that the calculations be made self-consistent with respect to both the calculated free magnesium-ion concentration and the ionic strength. This is a point which has been frequently neglected in earlier work in the literature. Nevertheless, this investigation has built upon the ideas of the earlier studies.

The use of such a model will be illustrated in this paper by application to the following reaction:

$$2\,\Sigma\mathrm{ADP(aq)} = \Sigma\mathrm{AMP(aq)} + \Sigma\mathrm{ATP(aq)} \qquad \text{(A)}$$

The abbreviations used here are: ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; and ATP, adenosine 5′-triphosphate; the suffix (aq) indicates that the reaction occurs in an aqueous solution of unspecified concentration. The Σ's denote the total amounts of each of these substances which may exist in a multiplicity of proton or metal bound forms in solution. When writing the reaction as above it is understood that protons and metal ions (e.g. magnesium ion) are involved in the reaction. Thus, a more complete way of writing this reaction is:

$$2\,\Sigma\mathrm{ADP(aq)} = \Sigma\mathrm{AMP(aq)} + \Sigma\mathrm{ATP(aq)} + \nu_{\mathrm{H}}\mathrm{H^+(aq)} + \nu_{\mathrm{Mg}}\mathrm{Mg^{2+}(aq)} \qquad \text{(B)}$$

The quantity ν_{H} is the stoichiometric number of protons produced as a consequence of the reaction. It may be either positive or negative and it is not necessarily an integer. Similarly, ν_{Mg} is the stoichiometric number of magnesium ions produced or absorbed. Although electrical charges have not been placed on the nucleotides, they are considered to be implicitly included. Thus, the reaction is viewed as being electrically balanced.

When these nucletotides have fully ionized hydroxyl groups, they will be referred to as $\mathrm{AMP^{2-}}$, $\mathrm{ADP^{3-}}$, and $\mathrm{ATP^{4-}}$, respectively. Proton and metal-ion bound forms will be indicated by the addition of an appropriate number of hydrogen or metal ions to these species, e.g. $\mathrm{H_2ATP^{2-}}$ and $\mathrm{MgHATP^{-}}$. All thermodynamic properties that refer to these species will be macroscopic properties and no attempt will be made to deal with microscopic properties that involve molecularly distinguishable ionic forms of these molecules.

Reaction (A) is catalyzed by the enzyme adenylate kinase (EC 2.7.4.3). This enzyme is also referred to as myokinase or ATP : AMP phosphotransferase. It was first isolated from rabbit muscle by Colowick and Kalckar in 1943 [5]. It has subsequently been found in a wide variety of biological media including mitochondria, liver, red blood cells, eye lens, and plants such as lemons. The reaction it catalyzes is recognized as one of the more significant reactions in the conversion of chemical to mechanical energy and in the control of metabolic reactions. A general review on this enzyme has been written by Noda [6]. Interest in the thermodynamics of this disproportionation reaction has been spurred by the fact that the concentration of the nucleotides *in vivo* is largely determined by the equilibrium constant for this reaction.

Thus, this system has been the subject of sixteen prior studies and equilibrium constants have been determined under a wide variety of conditions of temperature, pH, ionic strength, and magnesium ion concentration. Examination of these data indicated large discrepancies between the results of the various investigations. We therefore undertook a detailed equilibrium and calorimetric investigation of this reaction, the results of which are reported in the accompanying paper [7] along with a comparison with earlier results from the literature.

2. Description of model

The first step in the development of a model for the ADP disproportionation reaction system is the selection of a reference reaction. We have adopted the following one:

$$2\,\mathrm{ADP^{3-}(ao)} = \mathrm{AMP^{2-}(ao)} + \mathrm{ATP^{4-}(ao)} \qquad \text{(C)}$$

The standard equilibrium constant for this reference reaction will be denoted as K°_{ref}. It is given by:

$$K^\circ_{\mathrm{ref}} = a(\mathrm{AMP^{2-}})a(\mathrm{ATP^{4-}})/[a(\mathrm{ADP^{3-}})]^2 \qquad (1)$$

Here, the a's are the activities of the indicated ionic forms of the nucleotides. The standard state for the solute species is the hypothetical ideal solution of unit molality and the standard state for the solvent is the pure solvent. The standard state pressure is 0.1 MPa and the standard state temperature 298.15 K. The activities, activity coefficients, and the standard equilibrium constants are dimensionless. A solute species in this standard state is denoted by the suffix (ao), and thermodynamic parameters associated with this standard state are denoted by a superscript °. The reference reaction itself is independent of pH. However, the prediction of the dependency of the equilibrium constant for the overall reaction (A) on the pH and metal-ion concentrations will require thermodynamic data on the proton and metal-ion binding reactions involving ATP, ADP, and AMP. These reactions and the thermodynamic parameters associated with them are given in Table 1. The selection of the thermodynamic data given in this table is discussed later in this paper (see Section 3). Here, the heat capacity changes estimated by Alberty [8] have been used for many of the reactions in Table 1. Also, the parameters given in Table 1 have been obtained by adjustment of the measurements made in the several investigations to the thermochemical standard state with a common expression for the activity coefficients of the solute species (see eq. 32 below).

Application of mass balance considerations leads to the following:

$$\begin{aligned} m(\Sigma\mathrm{ADP}) = {} & m(\mathrm{ADP}^{3-}) + m(\mathrm{H_2ADP}^{2-}) \\ & + m(\mathrm{HADP}^{-}) + m(\mathrm{MgADP}^{-}) \\ & + m(\mathrm{MgHADP}) \end{aligned} \tag{2}$$

Introduction of equilibrium constants for the appropriate proton and magnesium binding reactions (see Table 1) leads to:

$$\begin{aligned} & m(\Sigma\mathrm{ADP}) \\ & = m(\mathrm{ADP}^{3-})\Big\{1 + m(\mathrm{H}^+)/[K_{1\mathrm{ADP}} m^\circ] \\ & \quad + [m(\mathrm{H}^+)]^2/\big[K_{1\mathrm{ADP}} K_{2\mathrm{ADP}} (m^\circ)^2\big] \\ & \quad + m(\mathrm{Mg}^{2+})/\big[K_{\mathrm{MgADP}} m^\circ\big] \\ & \quad + m(\mathrm{Mg}^{2+}) m(\mathrm{H}^+) \\ & \quad /\big[K_{\mathrm{MgHADP}} K_{1\mathrm{ADP}} (m^\circ)^2\big]\Big\} \end{aligned} \tag{3}$$

Table 1

Thermodynamic parameters at 298.15 K and 0.1 MPa relevant to the disproportionation of ADP to AMP and ATP in aqueous solution. The thermodynamic data for the reference reaction for this reaction are based upon our study [7]. The other parameters are the averages of the values given in the next to last column in Table 3 with the exception of those results indicated by a dagger (†). The uncertainties given for the pK's and enthalpy changes are equal to two standard deviations. The entropies have been calculated from the equilibrium constants and the enthalpy changes. The heat capacity changes for the proton and magnesium-ion binding reactions are estimated quantities [8]. The standard state is the hypothetical ideal solution of unit molality

Reaction	Equilibrium constant or pK	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔC_p°
$2\ \mathrm{ADP}^{3-} \rightleftharpoons \mathrm{ATP}^{4-} + \mathrm{AMP}^{2-}$	$K^\circ_{\mathrm{ref}} = 0.225 \pm 0.010$	-1.5 ± 1.5	-17 ± 5	≈ -46
$\mathrm{HAMP}^- \rightleftharpoons \mathrm{H}^+ + \mathrm{AMP}^{2-}$	$\mathrm{p}K^\circ_{1\mathrm{AMP}} = 6.74 \pm 0.07$	-5.4 ± 1.7	-147 ± 6	-126
$\mathrm{H_2AMP} \rightleftharpoons \mathrm{H}^+ + \mathrm{HAMP}^-$	$\mathrm{p}K^\circ_{2\mathrm{AMP}} = 3.99 \pm 0.05$	18.0 ± 1.8	-16 ± 6	-63
$\mathrm{MgAMP} \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{AMP}^{2-}$	$\mathrm{p}K^\circ_{\mathrm{MgAMP}} = 2.81 \pm 0.08$	-11.4 ± 3.8	-92 ± 13	-251
$\mathrm{HADP}^{2-} \rightleftharpoons \mathrm{H}^+ + \mathrm{ADP}^{3-}$	$\mathrm{p}K^\circ_{1\mathrm{ADP}} = 7.20 \pm 0.10$	-5.6 ± 1.6	-157 ± 6	-126
$\mathrm{H_2ADP}^- \rightleftharpoons \mathrm{H}^+ + \mathrm{HADP}^{2-}$	$\mathrm{p}K^\circ_{2\mathrm{ADP}} = 4.37 \pm 0.06$	17.6 ± 1.4	-25 ± 5	-63
$\mathrm{MgADP}^- \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{ADP}^{3-}$	$\mathrm{p}K^\circ_{\mathrm{MgADP}} = 4.68 \pm 0.14$	-19.0 ± 1.6	-153 ± 6	-188
$\mathrm{MgHADP} \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{HADP}^{2-}$	$\mathrm{p}K^\circ_{\mathrm{MgHADP}} = 2.52 \pm 0.09$	-12.5 ± 9.0	-90 ± 30	-251
$\mathrm{HATP}^{3-} \rightleftharpoons \mathrm{H}^+ + \mathrm{ATP}^{4-}$	$\mathrm{p}K^\circ_{1\mathrm{ATP}} = 7.62 \pm 0.09$	-6.3 ± 1.2	-167 ± 4	-126
$\mathrm{H_2ATP}^{2-} \rightleftharpoons \mathrm{H}^+ + \mathrm{HATP}^{3-}$	$\mathrm{p}K^\circ_{2\mathrm{ATP}} = 4.70 \pm 0.05$	14.9 ± 1.2	-40 ± 4	-63
$\mathrm{MgATP}^{2-} \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{ATP}^{4-}$	$\mathrm{p}K^\circ_{\mathrm{MgATP}} = 6.22 \pm 0.16$	-23.0 ± 1.8	-196 ± 7	-126
$\mathrm{MgHATP}^- \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{HATP}^{3-}$	$\mathrm{p}K^\circ_{\mathrm{MgHATP}} = 3.65 \pm 0.17$	-16.9 ± 8.3	-127 ± 28	-188
$\mathrm{Mg_2ATP} \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{MgATP}^{2-}$	$\mathrm{p}K_{\mathrm{Mg2ATP}} = 2.72 \pm 0.22$	-10.9 ± 1.5	-89 ± 7	-251

and

$$\begin{aligned} f(\mathrm{ADP}^{3-}) &= m(\mathrm{ADP}^{3-})/m(\Sigma\mathrm{ADP}) \\ &= \Big\{1 + m(\mathrm{H}^+)/[K_{1\mathrm{ADP}}m^\circ] + [m(\mathrm{H}^+)]^2 \\ &\quad /\big[K_{1\mathrm{ADP}}K_{2\mathrm{ADP}}(m^\circ)^2\big] \\ &\quad + m(\mathrm{Mg}^{2+})/\big[K_{\mathrm{MgADP}}m^\circ\big] \\ &\quad + m(\mathrm{Mg}^{2+})m(\mathrm{H}^+) \\ &\quad /\big[K_{\mathrm{MgHADP}}K_{1\mathrm{ADP}}(m^\circ)^2\big]\Big\}^{-1} \end{aligned} \tag{4}$$

Here, $f(\mathrm{ADP}^{3-})$ is defined as the fraction of the total ADP in the form ADP^{3-}. The standard molality (m°), equal to 1 mol kg^{-1}, is included since the equilibrium constants are dimensionless. Expressions for the fractions of ATP and AMP in the forms ATP^{4-} and AMP^{2-}, respectively, can also be derived. The results are:

$$\begin{aligned} f(\mathrm{ATP}^{4-}) &= m(\mathrm{ATP}^{4-})/m(\Sigma\mathrm{ATP}) \\ &= \Big\{1 + m(\mathrm{H}^+)/[K_{1\mathrm{ATP}}m^\circ] \\ &\quad + [m(\mathrm{H}^+)]^2/\big[K_{1\mathrm{ATP}}K_{2\mathrm{ATP}}(m^\circ)^2\big] \\ &\quad + m(\mathrm{Mg}^{2+})/\big[K_{\mathrm{MgATP}}m^\circ\big] \\ &\quad + \big[m(\mathrm{Mg}^{2+})\big]^2/\big[K_{\mathrm{MgATP}}K_{\mathrm{Mg2ATP}}(m^\circ)^2\big] \\ &\quad + m(\mathrm{Mg}^{2+})m(\mathrm{H}^+) \\ &\quad /\big[K_{\mathrm{MgHATP}}K_{1\mathrm{ATP}}(m^\circ)^2\big]\Big\}^{-1} \end{aligned} \tag{5}$$

$$\begin{aligned} f(\mathrm{AMP}^{2-}) &= m(\mathrm{AMP}^{2-})/m(\Sigma\mathrm{AMP}) \\ &= \Big\{1 + m(\mathrm{H}^+)/[K_{1\mathrm{AMP}}m^\circ] \\ &\quad + \big[m(\mathrm{Mg}^{2+})\big]^2/\big[K_{\mathrm{MgATP}}K_{\mathrm{Mg2ATP}}(m^\circ)^2\big] \\ &\quad + [m(\mathrm{H}^+)]^2/\big[K_{1\mathrm{AMP}}K_{2\mathrm{AMP}}(m^\circ)^2\big] \\ &\quad + m(\mathrm{Mg}^{2+})/\big[K_{\mathrm{MgAMP}}m^\circ\big]\Big\}^{-1} \end{aligned} \tag{6}$$

In a similar manner, one can calculate the fractions of the other species present in solution, i.e. $f(\mathrm{HATP}^{3-})$, $f(\mathrm{MgATP}^{2-})$, $f(\mathrm{MgHATP}^-)$, etc. Alberty [9] has recently used the symbol Z here rather than f. He also refers to this quantity as a partition function in analogy to molecular partition functions. Since this quantity is a mole fraction, the symbol x could also be used.

The equilibrium constants used in eqs. (3)–(6) are defined in terms of the appropriate molality ratios. For example, for the dissociation of $\mathrm{MgATP}^{2-}(\mathrm{aq})$:

$$\begin{aligned} K_{\mathrm{MgATP}} &= m(\mathrm{Mg}^{2+})m(\mathrm{ATP}^{4-}) \\ &\quad /\big[m(\mathrm{MgATP}^{2-})m^\circ\big] \end{aligned} \tag{7}$$

This equilibrium constant is related to the standard equilibrium constant (K°_{MgATP}) by the following:

$$\begin{aligned} K^\circ_{\mathrm{MgATP}} &= \big\{m(\mathrm{Mg}^{2+})m(\mathrm{ATP}^{4-}) \\ &\quad /\big[m(\mathrm{MgATP}^{2-})m^\circ\big]\big\} \\ &\quad \times\big\{\big[\gamma(\mathrm{Mg}^{2+})\gamma(\mathrm{ATP}^{4-})\big] \\ &\quad /\gamma(\mathrm{MgATP}^{2-})\big\} \end{aligned} \tag{8}$$

$$K^\circ_{\mathrm{MgATP}} = K_{\mathrm{MgATP}}\Gamma \tag{9}$$

Here, Γ is the activity coefficient ratio defined by the right most term in braces in eq. (8).

The quantity that is generally measured and reported in equilibrium investigations is:

$$K'_{\mathrm{A}} = m(\Sigma\mathrm{AMP})m(\Sigma\mathrm{ATP})/\big[m(\Sigma\mathrm{ADP})\big]^2 \tag{10}$$

It is the equilibrium ratio or apparent equilibrium constant as determined from measurements which yield the total amounts of the nucleotides ATP, AMP, and ADP in solution. It has frequently been referred to as K_{obs} in the literature [1–4]. Introduction of eqs. (4)–(6) into eq. (10) leads to:

$$\begin{aligned} K'_{\mathrm{A}} &= \big\{m(\mathrm{AMP}^{2-})m(\mathrm{ATP}^{4-})/\big[m(\mathrm{ADP}^{3-})\big]^2\big\} \\ &\quad \times\big\{\big[f(\mathrm{ADP}^{3-})\big]^2 \\ &\quad /\big[f(\mathrm{AMP}^{2-})f(\mathrm{ATP}^{4-})\big]\big\} \end{aligned} \tag{11}$$

$$\begin{aligned} K'_{\mathrm{A}} &= K_{\mathrm{ref}}\big\{\big[f(\mathrm{ADP}^{3-})\big]^2 \\ &\quad /\big[f(\mathrm{AMP}^{2-})f(\mathrm{ATP}^{4-})\big]\big\} \end{aligned} \tag{12}$$

The enthalpy and heat capacity changes for reaction (A), $\Delta H'_{\mathrm{A}}$ and $\Delta C'_{p,\mathrm{A}}$, can be obtained from the temperature derivatives of eq. (12):

$$\Delta H'_{\mathrm{A}} = RT^2(\partial \ln K'_{\mathrm{A}}/\partial T)_{p,n_i} \tag{13}$$

$$\begin{aligned}
\Delta H'_{\mathrm{A}} = {} & \Delta H_{\mathrm{ref}} + 2f(\mathrm{ADP}^{3-})\Big\{ m(\mathrm{Mg}^{2+})\Delta H_{\mathrm{MgADP}} \\
& /(K_{\mathrm{MgADP}} m^{\circ}) \\
& + m(\mathrm{H}^+)\Delta H_{1\mathrm{ADP}}/(K_{1\mathrm{ADP}} m^{\circ}) \\
& + \Big[m(\mathrm{H}^+) m(\mathrm{Mg}^{2+}) \\
& /(K_{1\mathrm{ADP}} K_{\mathrm{MgHADP}})(m^{\circ})^2 \Big] \\
& \times (\Delta H_{1\mathrm{ADP}} + \Delta H_{\mathrm{MgHADP}}) \\
& + \Big[(m(\mathrm{H}^+))^2/\big(K_{1\mathrm{ADP}} K_{2\mathrm{ADP}} (m^{\circ})^2\big) \Big] \\
& \times (\Delta H_{1\mathrm{ADP}} + \Delta H_{2\mathrm{ADP}}) \Big\} \\
& - f(ATP^{4-}) \Big\{ m(Mg^{2+})\Delta H_{MgATP} \\
& /(K_{MgATP} m^{\circ}) + m(H^+)\Delta H_{1ATP} \\
& /(K_{1ATP} m^{\circ}) + \Big[m(H^+) m(Mg^{2+}) \\
& /\big(K_{1ATP} K_{MgHATP} (m^{\circ})^2\big) \Big] \\
& \times (\Delta H_{1ATP} + \Delta H_{MgHATP}) \\
& + \Big[(m(H^+))^2/\big(K_{1ATP} K_{2ATP} (m^{\circ})^2\big) \Big] \\
& \times (\Delta H_{1ATP} + \Delta H_{2ATP}) + \Big[(m(Mg^{2+}))^2 \\
& /\big(K_{MgATP} K_{Mg2ATP} (m^{\circ})^2\big) \Big] \\
& \times (\Delta H_{MgATP} + \Delta H_{Mg2ATP}) \Big\} \\
& - f(AMP^{2-}) \Big\{ m(Mg^{2+})\Delta H_{MgAMP} \\
& /(K_{MgAMP} m^{\circ}) + m(H^+)\Delta H_{1AMP} \\
& /(K_{1AMP} m^{\circ}) + \Big[(m(H^+))^2 \\
& /\big(K_{1AMP} K_{2AMP} (m^{\circ})^2\big) \Big] \\
& \times (\Delta H_{1AMP} + \Delta H_{2AMP}) \Big\}
\end{aligned} \tag{14}$$

$$\Delta C'_{p,\mathrm{A}} = (\partial\, \Delta H'_{\mathrm{A}}/\partial T)_p \tag{15}$$

The derivative which can be obtained from eq. (15) is particularly cumbersome. Its value, however, is more easily determined by calculating $\Delta H'_{\mathrm{A}}$ at several different temperatures and taking the tangent to the curve in a plot of ΔH as a function of temperature. Alberty [8] has given explicit terms for the heat capacity change for the hydrolysis of ATP which can be applied, with appropriate modifications to the disproportionation reaction of ADP.

We now consider a mixture containing 1 kg of water and n°_{ADP} moles of ADP, n°_{AMP} moles of AMP, and n°_{ATP} moles of ATP at the start of the reaction. At equilibrium the amounts of the various nucleotides will be given by:

$$n_{\mathrm{ADP}} = n^{\circ}_{\mathrm{ADP}} + \nu_{\mathrm{ADP}}\xi \tag{16}$$

$$n_{\mathrm{AMP}} = n^{\circ}_{\mathrm{AMP}} + \nu_{\mathrm{AMP}}\xi \tag{17}$$

$$n_{\mathrm{ATP}} = n^{\circ}_{\mathrm{ATP}} + \nu_{\mathrm{ATP}}\xi \tag{18}$$

Here the stoichiometric numbers (ν) of ADP, AMP and ATP are, respectively, equal to -2, $+1$, and $+1$. The apparent equilibrium constant K'_{A} can be expressed in terms of the extent of reaction (ξ):

$$\begin{aligned}
K'_{\mathrm{A}} = {} & (n^{\circ}_{\mathrm{AMP}} + \nu_{\mathrm{AMP}}\xi)(n^{\circ}_{\mathrm{ATP}} + \nu_{\mathrm{ATP}}\xi) \\
& /(n^{\circ}_{\mathrm{ADP}} + \nu_{\mathrm{ADP}}\xi)^2
\end{aligned} \tag{19}$$

If K'_{A} and the initial stoichiometric amounts of substances are known, eq. (19) can be solved for ξ. Then, from eqs. (16)–(18), the total amounts of each of the nucleotides at equilibrium can be calculated. The molalities of all of the species in solution can be obtained from the calculated values of the fractions of each of the species in solution (eqs. 4–6 and similar ones for the other species). For example:

$$m(\mathrm{ATP}^{4-}) = f(\mathrm{ATP}^{4-})\, m(\Sigma\mathrm{ATP}) \tag{20}$$

The extent of reaction is also useful in calculations involving calorimetric measurements. Thus, the calorimetric heat of reaction for (A) is:

$$q = \xi\, \Delta H'_{\mathrm{A}} \tag{21}$$

If there is a reaction between the protons or magnesium ions produced (or absorbed) and any other solutes in the solution, it is necessary to include a contribution to the heat from these reactions. For example, for the protonation of a buffer, the heat of reaction is given by:

$$q = -n_{\mathrm{H}}\, \Delta_{\mathrm{ion}} H \tag{22}$$

Here, $\Delta_{\mathrm{ion}}H$ is the enthalpy of ionization of the appropriate buffer. Thus, if both the heat of reaction and the extent of reaction are measured (or calculated from eq. 19), the enthalpy of reaction $\Delta H'_{\mathrm{A}}$ is determined. In favorable cases calorimetry can also be used to determine the equilibrium constant. Thus, if an enthalpy measurement is performed in which only ADP is initially present in solution, i.e. from the forward direction, the heat of reaction for a solution containing one kilogram of water and neglecting any additional reactions such as buffer protonation is:

$$q_{\mathrm{for}} = \xi_{\mathrm{for}}\ \Delta H'_{\mathrm{A}} \tag{23}$$

Similarly, if only AMP and ATP are initially present in solution, i.e. from the reverse direction, the heat of reaction for a solution containing one kilogram of water is:

$$q_{\mathrm{rev}} = -\xi_{\mathrm{rev}}\ \Delta H'_{\mathrm{A}} \tag{24}$$

Equation (19) can be used to generate two additional equations involving the known stoichiometric amounts of the nucleotides and the extents of reaction from the two different directions of the reaction. Thus, there are now four equations with four unknowns ($\Delta H'_{\mathrm{A}}$, K'_{A}, ξ_{for}, and ξ_{rev}) which can then be solved for these desired quantities. This methodology was applied in 1941 by Sturtevant [10] in a study of the mutarotation of aqueous glucose. An alternative procedure is to vary the composition of the initial reaction mixture so as to determine the composition of the mixture which produces no enthalpy of reaction. If the enthalpy of reaction is not equal to zero and if there is no enthalpy of interaction of the enzyme with the reactants or products, a knowledge of the composition of that reaction mixture allows one to directly calculate the apparent equilibrium constant. This type of procedure was used by Benzinger and Hems [11] in their study of the reaction catalyzed by glutaminase (EC 3.5.1.2).

Information on the stoichiometry of the reaction can also be obtained with the relationship:

$$\nu_X = -\left[\partial \ln K'_{\mathrm{A}}/\partial \ln m(X)\right]_{T,p,j \neq X} \tag{25}$$

Here, X is any ligand which participates in the overall reaction, e.g. the hydrogen and magnesium ions for the reaction being studied herein; j is any of the other solutes in solution. This equation, given by Alberty [1,9] and attributed by him to Wyman [12], is based upon a generalized relationship giving the amount of ligand bound per amount of substance binding the ligand (see eq. 9.31 in ref. [9]). Note that Alberty's eq. (9.59) in ref. [9] is opposite in sign to eq. (25) above since $\Delta\nu_{\mathrm{H}}$ in his equation refers to the change in binding of the hydrogen ion by the substances binding it. Also, in eq. (10) in ref. [1], the pH is based on the concentration (mol dm^{-3}) and not the activity of the hydrogen ion. Use of eqs. (4)–(6), (12), and (25) leads to:

$$\begin{aligned}\nu_{\mathrm{H}} = {} & 2\Big\{\left[m(\mathrm{H}^+)/(K_{1\mathrm{ADP}}m^\circ)\right]\left(1 + m(\mathrm{Mg}^{2+})\right.\\ & \left./(K_{\mathrm{MgHADP}}m^\circ)\right) + 2\left[m(\mathrm{H}^+)\right]^2\\ & /\left(K_{1\mathrm{ADP}}K_{2\mathrm{ADP}}(m^\circ)^2\right)\Big\}f(\mathrm{ADP}^{3-})\\ & -\Big\{\left[m(\mathrm{H}^+)/(K_{1\mathrm{ATP}}m^\circ)\right]\left(1 + m(\mathrm{Mg}^{2+})\right.\\ & \left./(K_{\mathrm{MgHATP}}m^\circ)\right) + 2\left[m(\mathrm{H}^+)\right]^2\\ & /\left(K_{1\mathrm{ATP}}K_{2\mathrm{ATP}}(m^\circ)^2\right)\Big\}f(\mathrm{ATP}^{4-})\\ & -\Big\{m(\mathrm{H}^+)/(K_{1\mathrm{AMP}}m^\circ) + 2\left[m(\mathrm{H}^+)\right]^2\\ & /\left(K_{1\mathrm{AMP}}K_{2\mathrm{AMP}}(m^\circ)^2\right)\Big\}f(\mathrm{AMP}^{2-})\end{aligned} \tag{26}$$

Similarly, the use of eqs. (4)–(6), (12), and (25) leads to the following equation for the stoichiometric number of magnesium ions produced or absorbed:

$$\begin{aligned}\nu_{\mathrm{Mg}} = {} & 2\Big\{m(\mathrm{Mg}^{2+})/(K_{\mathrm{MgADP}}m^\circ)\\ & + m(\mathrm{H}^+)m(\mathrm{Mg}^{2+})\\ & /(K_{\mathrm{MgHADP}}K_{1\mathrm{ADP}})(m^\circ)^2\Big\}f(\mathrm{ADP}^{3-})\\ & -\Big\{m(\mathrm{Mg}^{2+})/(K_{\mathrm{MgATP}}m^\circ)\\ & + 2\left[m(\mathrm{Mg}^{2+})\right]^2\\ & /\left(K_{\mathrm{MgATP}}\mathrm{K}_{\mathrm{Mg2ATP}}(m^\circ)^2\right)\\ & + m(\mathrm{H}^+)m(\mathrm{Mg}^{2+})\\ & /\left(K_{\mathrm{MgHATP}}K_{1\mathrm{ATP}}(m^\circ)^2\right)\Big\}f(\mathrm{ATP}^{4-})\\ & -\left\{m(\mathrm{Mg}^{2+})/(K_{\mathrm{MgAMP}}m^\circ)\right\}f(\mathrm{AMP}^{2-})\end{aligned} \tag{27}$$

The quantity n_H can in principle be determined from a direct measurement of the amount of acid or base required to maintain the reaction mixture at a constant pH. If the extent of reaction (ξ) is also known, the quantity ν_H can be calculated. This method has been used by Green and Mommaerts [13] to determine the variation of ν_H with pH, pMg, and pCa for the hydrolysis of ATP to ADP and inorganic phosphate and by Gellert and Sturtevant [14] in their study of the creatine kinase reaction.

The equations which have been developed are dependent upon a knowledge of the molalities of the free hydrogen and magnesium ions. Typically, one relies upon a pH measurement to determine the activity of the hydrogen ion and then calculates the molality of the free magnesium ions from the appropriate metal-ion binding constants (see Table 1). This latter calculation is readily performed with a material balance involving all of the magnesium species:

$$\begin{aligned} m(\Sigma\mathrm{Mg}) = {} & m(\mathrm{Mg}^{2+}) + m(\mathrm{MgATP}^{2-}) \\ & + 2m(\mathrm{Mg_2ATP}) + m(\mathrm{MgHATP}\ \ ) \\ & + m(\mathrm{MgADP}^{-}) \\ & + m(\mathrm{MgHADP}) + m(\mathrm{MgAMP}) \end{aligned} \tag{28}$$

Introduction of appropriate equilibrium constants into eq. (28) leads to:

$$\begin{aligned} & m(\mathrm{Mg}^{2+}) \\ & = m(\Sigma\mathrm{Mg})\Big\{1 + \big[f(\mathrm{ATP}^{4-})m(\Sigma\mathrm{ATP}) \\ & \quad /(K_{\mathrm{MgATP}}m^{\circ})\big] \\ & \quad + 2f(\mathrm{MgATP}^{2-})m(\Sigma\mathrm{ATP})/(K_{\mathrm{Mg2ATP}}m^{\circ}) \\ & \quad + f(\mathrm{HATP}^{3-})m(\Sigma\mathrm{ATP})/(K_{\mathrm{MgHATP}}m^{\circ}) \\ & \quad + f(\mathrm{ADP}^{3-})m(\Sigma\mathrm{ADP})/(K_{\mathrm{MgADP}}m^{\circ}) \\ & \quad + f(\mathrm{HADP}^{2-})m(\Sigma\mathrm{ADP})/(K_{\mathrm{MgHADP}}m^{\circ}) \\ & \quad + f(\mathrm{AMP}^{2-})m(\Sigma\mathrm{AMP})/(K_{\mathrm{MgAMP}}m^{\circ})\Big\}^{-1} \end{aligned} \tag{29}$$

Note that the application of eq. (29) is dependent upon a knowledge of the fractions of the various species in solution which, in turn, are dependent upon a knowledge of the molality of the free magnesium ion. Both this calculation and others require iterative procedures to obtain self-consistent solutions. This matter is dealt with below. Also note that an assumption is involved in obtaining the activity of the hydrogen ion from a pH measurement, namely that pH is equal to $-\log a(\mathrm{H}^+)$. Since the current pH scale is an operational one [15], this relationship must be considered to be an approximation to be used in order to make reasonable progress. However, if this treatment is used solely as an extrapolation device in the calculation of thermodynamic properties, any errors introduced caused by making this assumption should be minimized. In principle, one might also use a specific-ion electrode to determine the molality of the free magnesium ion. However, our experience and that of others [16] with this type of electrode has shown that it is subject to substantial interferences from other ions in solution. Thus, it cannot be used to obtain a reliable value for the molality of the free magnesium ion.

When performing calculations it will frequently be necessary to adjust Gibbs energies, equilibrium constants, and enthalpies from a reference temperature (θ) to another temperature (T). The relationships which we have used for this purpose are:

$$\begin{aligned} \Delta G_T^{\circ} & = -RT \ln K_T^{\circ} \\ & = \Delta H_\theta^{\circ} + \Delta C_p^{\circ}(T-\theta) + T(\Delta G_\theta^{\circ} - \Delta H_\theta^{\circ}) \\ & \quad /\theta - T\Delta C_p^{\circ} \ln(T/\theta) \end{aligned} \tag{30}$$

$$\Delta H_T^{\circ} = \Delta H_\theta^{\circ} + \Delta C_p^{\circ}(T-\theta) \tag{31}$$

For biochemical systems, a problem that is generally faced is the lack of activity coefficient data, particularly those appropriate to mixtures containing both electrolytes and non-electrolytes. The expression which we have used [17,18] for the estimation of the activity coefficients of a species i with charge z_i is:

$$\ln \gamma_i = -A_m z_i^2 I^{1/2}/(1 + BI^{1/2}) + 2\lambda\Sigma_j m_j \tag{32}$$

This is an extended Debye-Hückel equation in which the pairwise-interaction parameters (λ_{ij}) are assumed to have a constant value equal to λ and m_j is the molality of the jth species. The ionic strength (I) is equal to $\frac{1}{2}\Sigma_i m_i z_i^2$. Values of the Debye-Hückel constant (A_m) and its temperature derivatives have been tabulated in the literature [19–21]. The pairwise-interaction parameter appears to have first been used together with a Debye-Hückel expression by Guggenheim [22]. It was also used only slightly later by Scatchard [23] in treating the thermodynamic properties of strong electrolytes. In previous calculations we have used an estimated value of 1.6 $\text{kg}^{1/2}$ $\text{mol}^{-1/2}$ for B based upon the values of this parameter obtained in fitting data on a series of electrolytes of charge type 1–1, 1–2, and 2–1 [24–29]. The same value for B was also recommended by Vasil'ev [30] based upon the earlier work of Vanderzee and Dawson [31]. However, in fitting the available proton and metal-ion binding constants needed for the treatment of the adenylate kinase equilibrium data (see Section 3), a value of 4.0 $\text{kg}^{1/2}$ $\text{mol}^{-1/2}$ was obtained for B. Note that a value for λ could also be introduced into the calculations if the data justify it. In fact, one could attempt a regression of values for B and/or λ from a set of sufficiently precise equilibrium data for the reaction in which systematic variations in ionic strength and overall composition were performed. In the description of the thermodynamics of a system, a negative value of λ_{ij} is equivalent to an association while a positive value corresponds to a repulsive interaction between species i and j.

If activity coefficient data are available for the components of the reaction mixture, this information can be used in the calculations in conjunction with the Pitzer formalism [32]. The full Pitzer equations for activity coefficients in mixed electrolytes also require the θ and ψ terms which are used to account for short range interactions. Millero [33,34] has described the application of this formalism to calculations in sea and natural waters.

Note that the calculation of the activity coefficients of the species in solution requires a knowledge of the ionic strength and the composition of the solution at equilibrium which are also objects of the actual calculation. Thus, as in the case of the calculation of the molality of the free magnesium ion, $m(\text{Mg}^{2+})$, it is necessary to introduce iterative procedures to make the overall calculation self-consistent. It is possible to raise fundamental objections to the use of single-ion activity coefficients [35]. Nevertheless, the use of these quantities can be justified since those properties which we are most interested in calculating (K'_A and $\Delta H'_A$) are measurable quantities. Thus, the single-ion activity coefficients, γ_i, and the B and λ terms can be viewed solely as parameters in a model which is used to calculate measurable quantities.

Similarly, it is necessary to calculate enthalpies for reactions under actual reaction conditions (ΔH) from standard enthalpy changes (ΔH°). Here, the appropriate relationship is:

$$\Delta H = \Delta H^\circ + \Delta H^{\text{ex}} \tag{33}$$

Here, H_i^{ex} is the excess enthalpy of species i in the solution. The Δ's refer to the differences obtained from the appropriate stoichiometric combination of the enthalpies of the reactants and products for a given reaction. The quantity H_i^{ex} is obtained by taking the temperature derivative of the excess Gibbs energy of species i:

$$G_i^{\text{ex}} = RT \ln \gamma_i = -A_m RT z_i^2 I^{1/2}/(1 + BI^{1/2}) + 2RT\lambda\Sigma_j m_j \tag{34}$$

Thus, assuming that the temperature dependency of B and λ are zero:

$$\begin{aligned} S_i^{\text{ex}} &= -(\partial G_i^{\text{ex}}/\partial T)_{p,j\neq i} \\ &= A_m R z_i^2 I^{1/2}/(1 + BI^{1/2}) \\ &\quad + (\partial A_m/\partial T)_p RT z_i^2 I^{1/2}/(1 + BI^{1/2}) \\ &\quad - 2R\lambda\Sigma_j m_j \end{aligned} \tag{35}$$

$$H_i^{\text{ex}} = G_i^{\text{ex}} + TS_i^{\text{ex}} = (\partial A_m/\partial T)_p RT^2 z_i^2 I^{1/2} /(1 + BI^{1/2}) \tag{36}$$

It is also possible to calculate the excess Gibbs energy (G_1^{ex}) and the activity of the water a_1 from eq. (32) and the Gibbs-Duhem equation applied to the Gibbs energy. This leads to:

$$G_1^{\text{ex}} = 2A_{\text{m}}RT\sigma/(m_1^* B^3) - RT\lambda(\Sigma_j m_j)^2/m_1^* \tag{37}$$

$$\ln(a_1) = G_1^{\text{ex}}/RT - \Sigma_j m_j/m_1^* \tag{38}$$

Here, m_1^* is the amount of water in a kilogram of water (55.5084 mol) and the quantity σ is defined by:

$$\sigma = (1 + BI^{1/2}) - 2\ln(1 + BI^{1/2}) - (1 + BI^{1/2})^{-1} \tag{39}$$

The term $(1 + BI^{1/2})$ must be kept positive so that the second term on the right side of eq. (39) remains a mathematically real quantity. Application of the Gibbs–Duhem equation for the enthalpy and eq. 36 leads to an expression for the excess enthalpy of the water:

$$H_1^{\text{ex}} = -2RT^2\sigma(\partial A_{\text{m}}/\partial T)_p/(m_1^* B^3) \tag{40}$$

Equations (37)–(40) are needed if the water is a participant in any of the equilibria used to model the overall reaction in solution. While this is not the case for the reaction catalyzed by adenylate kinase, these relations are presented both for the sake of completeness and for application to other systems in which water is a participant in the reactions.

In the analysis of calorimetric experiments, one must consider effects attributable to the following enthalpy changes: the standard state enthalpy change for the reference reaction; enthalpies of proton and magnesium ion binding; the difference in enthalpies for reactants and products from the standard state to the actual experimental conditions; and the enthalpy of protonation of the buffer. Therefore, the molar enthalpy change for a calorimetric experiment is equal to:

$$\Delta H = \Delta H_{\text{ref}}^{\circ} + \Delta H_{\text{corr}} + \Delta H^{\text{ex}} - \nu_{\text{H}}\,\Delta H_{\text{ion}} \tag{41}$$

In the above equation: $\Delta H_{\text{ref}}^{\circ}$ is the standard state enthalpy change for the reference reaction; ΔH_{corr} is equal to the sum of all of the terms to the right of ΔH_{ref} in eq. (14) and is the correction for proton and magnesium ion binding; ΔH_i^{ex} is the difference between the enthalpy change for the reference reaction at the actual ionic strength of the solution and the standard state enthalpy change for that reaction (see eq. 33); and $\nu_{\text{H}}\,\Delta H_{\text{ion}}$ is the buffer protonation correction on a molar basis. Note that in calculating ΔH_{corr} from eq. (14) the various enthalpy changes for the proton and magnesium ion binding reactions pertain to the ionic strength of the actual solution. Thus, one must make use of eq. (33) to obtain the applicable values for these quantities.

Throughout, it has been assumed that the enzyme is present only in catalytic amounts and has no effect on the thermodynamics of any of the reactions. Thus, the enzyme is being viewed as an ideal catalyst which only serves to lower the energy of activation of the chemical reaction. If, however, the enzyme participates in any of the equilibria, e.g. binds to any of the reactants or products, a complete discussion of the thermodynamics would require the inclusion of the appropriate reaction equilibria in the overall formulation for the reacting system. One experimental test which can be performed to aid in resolving this issue, however, is to measure the heat associated with the mixing of a solution containing the enzyme with another solution containing the amounts of substrates present in the equilibrium mixture. If the measured heat is zero, this implies either that there is no binding of the enzyme with the substrates or cofactors, or that the enthalpy of binding is zero. The use of this test presupposes a prior knowledge of the composition of the equilibrium mixture. Also, as pointed out in the above discussion, this same method can be used, albeit with the assumption that there is no such interaction of the enzyme with the substrates or cofactors, to determine the composition of the equilibrium mixture and thereby the equilibrium constant. This method may not have sufficient sensitivity if the extent of reaction is large.

The above discussion contains the essential information needed to perform thermodynamic calculations on complex biochemical reactions such as the one catalyzed by adenylate kinase. The implementation of this information requires the use of a computational procedure which is summarized in Table 2 in the form of a flow chart.

Table 2

Flow chart for complex equilibrium calculations

Initial data required

1 K°, ΔH°, ΔC_p° at the reference temperature θ for each reaction in Table 1.
2 Activity coefficient parameters: B and λ.
3 Temperature, pH, and stoichiometric amounts of components in solution.
4 Initial estimates of I and pMg.

Calculate

1 K°, ΔH°, and ΔC_p° for each reaction in solution at the temperature of interest (eqs. 30 and 31).
2 Activity coefficients, excess enthalpies, and the activity of water (eqs. 32, 36, 38, and 40).
3 K and ΔH for each reaction in solution at the ionic strength and composition of the actual solution (eqs. 9 and 33). These values will be used in steps 4, 5, 7, and 13 below.
4 Fractions of species (eqs. 4–6 and similar ones for other species).
5 K'_A and ξ (eqs. 12 and 19, respectively).
6 Total amounts of reactants and products at equilibrium (eqs. 16–18).
7 The molality of the free magnesium ion, $m(\mathrm{Mg}^{2+})$ (eq. 29).
8 Test for convergence in $m(\mathrm{Mg}^{2+})$. Return to Step 4, if the convergence test is not passed.
9 Molalities of species (eq. 20 and similar ones for other species).
10 Ionic strength ($I = \frac{1}{2}\Sigma_j m_j^2$).
11 Test for convergence in the ionic strength. Return to Step 2 if the convergence test is not passed.
12 ν_H and ν_{Mg} (eqs. 26 and 27).
13 Final values of K'_A (eq. 12), $\Delta H'_A$ (eq. 14), and $\Delta C'_{p,A}$ (eq. 15).
14 Measured enthalpy change (eq. 41).

Note that it requires iterative procedures to assure self-consistency in both the molality of the free magnesium ion and the ionic strength (steps 8 and 11). A way of looking at what appears to be a lengthy computational procedure is to view it as a thermodynamic model which uses standard thermodynamic properties (K°, ΔH°, and ΔC_p°) for the ionic reactions occurring in solution together with a model for the activity coefficients of the species in solution (eq. 32) to calculate quantities which can be determined from experiments. These quantities include amounts of reactants and products, which yield values for K'_A and n_H, and measured molar enthalpy changes. The temperature and composition derivatives of these measured quantities also yield additional information as discussed above.

3. Selection of proton and metal-ion binding constants

The application of the model described above requires thermodynamic data (K°, ΔH°, and ΔC_p°) for the proton and metal-ion binding reactions that participate in the equilibria in solution. The selection of a set of such data for the adenylate kinase reaction is now considered. Table 3 contains a summary of these data from the literature. The format used in this table is similar to that used in previous compilations of proton and metal ion binding constants [36–39]. Included in Table 3 are data on these systems which were obtained from the references cited in these earlier compilations [36–39] and those papers located with a computer search of Chemical Abstracts. In all cases the original works were examined.

While there are several studies for each reaction, the ionic strength dependency of the equilibrium data was investigated in only a few cases [40–43]. These few data sets were used to obtain values of B which ranged from 0.8 to 11.2 $\mathrm{kg}^{1/2}$ $\mathrm{mol}^{-1/2}$. Here, we used the extended Debye–Hückel expression (eq. 32) for the activity coefficients of the ions in solution and with λ equal to zero. The average value was 5.4 $\mathrm{kg}^{1/2}$ $\mathrm{mol}^{-1/2}$. These values were obtained by performing a regression calculation of B for each data set. A value of $B = 1.5$ $\mathrm{kg}^{1/2}$ $\mathrm{mol}^{-1/2}$ was obtained from a preliminary set of values of the proton and

Table 3

Proton and magnesium-ion binding constants for AMP, ADP, and ATP and associated enthalpy changes. The presence of a question mark in this table indicates that the desired information is not contained in the reference(s) cited. The values in the next to last column are the results of the adjustment of the thermodynamic data, i.e. the measured p*K* 's and enthalpy changes, to the standard state (see Discussion in text). The averages of the values given in the next to last columns are given in Table 1. The uncertainties given there are equal to two standard deviations. Results indicated by a dagger (†) have not been included in these averages. An asterisk (*) indicates the author(s) extrapolation of the data to the standard state

A. $HAMP^-(aq) \rightleftharpoons H^+(aq) + AMP^{2-}(aq)$ (pK_{1AMP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	298.15	?	?	6.2	≈ 6.64	Wassermeyer [45]
pH	298.15	NaCl	0.15	6.05	6.55	Alberty et al. [46]
pH	298.15	NaCl	0.1	6.1	6.54	Bock et al. [47]
pH	293.15	KCl	0.1	6.14 ± 0.02	6.59	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	6.45 ± 0.02	7.00	Smith and Alberty [49]
pH	298.15	$(CH_3)_4NBr$	0.1	6.40	6.84	Tucci et al. [50]
	298.15	KCl	0.1	6.30	6.74	
pH	298.15	KNO_3	0.1	6.21	6.65	Taqui Khan and Martell [51]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	6.44 ± 0.01	6.88	Phillips et al. [41]
			0.0	6.67 ± 0.03 *	6.67	
pH	298.15	$NaClO_4$	0.1	6.14 ± 0.03	6.58	Sigel and Brintzinger [52]
pH	298.15	KNO_3	0.1	6.23 ± 0.02	6.67	Taqui Khan and Martell [53]
pH	288.15	KNO_3	0.1	6.25	6.71	Frey and Stuehr [54]
pH	298.15	KNO_3	0.1	6.21 ± 0.01	6.65	Banyasz and Stuehr [55]
pH	281.15	?	?	6.5	≈ 6.98	Jaffe and Cohn [56]
pH	298.15	?	0.2	6.37	≈ 6.92	Trimm and Patel [57]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	6.17 ± 0.20	≈ 6.79	Tate [58]
pH	298.15	$NaClO_4$	0.1	6.29 ± 0.02	6.73	Shanbhag and Choppin [59]
		$(C_2H_5)_4NCl$	0.1	6.46	6.90	

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH	ΔH°	Reference
				(kJ mol^{-1})		
Calorimetry	298.15	$(CH_3)_4NOH$	0.0	−7.5 ± 0.4 *	−7.5	Christensen and Izatt [60]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−3.65 ± 1.3 *	-3.6_5	Phillips et al. [41]
pH	298.15	KNO_3	0.1	+7.9 ± 0.4	+6.6 †	Taqui Khan and Martell [53]
pH	298.15	KNO_3	0.1	−4.6 ± 0.8	−5.9	Banyasz and Stuehr [55]
Calorimetry	298.15	$NaClO_4$	0.1	−3.34 ± 0.1	−4.6	Shanbhag and Choppin [59]

B. $H_2AMP(aq) \rightleftharpoons H^+(aq) + HAMP^-(aq)$ (pK_{2AMP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	296.15	?	?	3.8	≈ 4.00	Wassermeyer [45]
pH	298.15	NaCl	0.15	3.74	3.99	Alberty et al. [46]
pH	298.15	NaCl	0.1	3.7	3.92	Bock et al. [47]
pH	293.15	KCl	0.1	3.81 ± 0.01	3.97	Martell and Schwarzenbach [48]
pH	298.15	KNO_3	0.1	3.81	4.03	Taqui Khan and Martell [51]
pH	298.15	KNO_3	0.1	3.80 ± 0.01	4.02	Taqui Khan and Martell [53]
pH	288.15	KNO_3	0.1	3.96	4.18 †	Frey and Stuehr [54]
pH	298.15	KNO_3	0.1	3.82 ± 0.01	4.04	Banyasz and Stuehr [55]
Spectrophotometry	298.15	NaCl	0.1	3.84 ± 0.02	4.06	Ogasawara and Inoue [61]
pH	298.15	?	0.2	3.57	≈ 3.84 †	Trimm and Patel [57]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	≈ 3.68 ± 0.02	3.96	Tate [58]

Table 3 (continued)

B. $H_2AMP(aq) \rightleftharpoons H^+(aq) + HAMP^-(aq)$ (pK_{2AMP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH°	Reference
Calorimetry	298.15	$(CH_3)_4NOH$	0.0152	17.6 ± 0.4	17.3	Christensen and Izatt [60]
pH	298.15	KNO_3	0.1	20.5 ± 0.4	19.9	Taqui Khan and Martell [53]
pH	298.15	KNO_3	0.1	17.6 ± 0.8	17.0	Banyasz and Stuehr [55]

C. $MgAMP(aq) \rightleftharpoons Mg^{2+}(aq) + AMP^{2-}(aq)$ (pK_{MgAMP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	$pK^\circ_{298.15}$	Reference
pH	293.15	KCl	0.1	1.69 ± 0.02	2.59	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	1.69 ± 0.02	2.78	Smith and Alberty [62]
Resin	296.15	NaCl	0.1	2.00	2.89	Nanninga [63]
pH	298.15	KCl	0.1	2.14	3.02	Weitzel and Spehr [64]
Resin	296.15	NaCl	0.1	1.95	2.84	Walaas [65]
pH	298.15	KNO_3	0.1	1.97 ± 0.01	2.85	Taqui Khan and Martell [51]
pH	298.15	$NaClO_4$	0.1	1.63	2.51	Sigel and Brintzinger [52]
pH	298.15	KNO_3	0.1	1.97 ± 0.02	2.85	Taqui Khan and Martell [53]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	1.81	2.88	Belaich and Sari [66]
pH	288.15	KNO_3	0.1	1.80	2.73	Frey and Stuehr [54]
Regression	288.15	$(n\text{-}C_4H_9)_4NCl$	0.46	1.73 ± 0.06	3.15 †	De Weer and Lowe [67]
Calorimetry	298.15	KCl	0.1	1.80	2.68	Rialdi et al. [43]
pH	298.15	?	0.2	1.83	≈ 2.92	Trimm and Patel [57]
pH	298.15	$NaClO_4$	0.1	2.10	2.98	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1}	ΔH (kJ mol^{-1})	ΔH°	Reference
pH	298.15	KNO_3	0.1	−14.2 ± 0.8	−16.7	Taqui Khan and Martell [53]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	−7.4	−10.8	Belaich and Sari [66]
Calorimetry	298.15	KCl	0.1	−7.1	−10.8	Rialdi et al. [43]
Calorimetry	298.15	$NaClO_4$	0.1	−5.7 ± 0.1	−8.2	Shanbhag and Choppin [59]

D. $HADP^{2-}(aq) \rightleftharpoons H^+(aq) + ADP^{3-}(aq)$ (pK_{1ADP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	$pK^\circ_{298.15}$	Reference
pH	298.15	NaCl	0.15	6.26	7.01	Alberty et al. [46]
pH	298.15	$(C_2H_5)_4NBr$	0.2	6.65 ± 0.01	7.47	Melchior [68]
pH	298.15	NaCl	0.1	6.3	6.96	Bock et al. [47]
pH	293.15	KCl	0.1	6.35 ± 0.03	7.02	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	6.68 ± 0.02	7.50	Smith and Alberty [62]
pH	298.15	KCl	0.1	6.61	7.27	Weitzel and Spehr [64]
pH	303.15	$(C_2H_5)_4NBr$	0.1	6.65	7.30	O'Sullivan and Perrin [69]
pH	298.15	$(CH_3)_4NBr$	0.0	7.00 *	7.00	Izatt and Christensen [40]
pH	298.15	KNO_3	0.1	6.44	7.10	Taqui Khan and Martell [51]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	6.78 ± 0.01	7.44	Phillips et al. [41,42]
			0.0	7.20 ± 0.04 *	7.20	
pH	298.15	KNO_3	0.1	6.44 ± 0.02	7.10	Taqui Khan and Martell [53]
pH	281.15	?	?	6.8	≈ 7.50	Jaffe and Cohn [56]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	6.28 ± 0.04	≈ 6.94	Tate [58]
NMR	303.15	KNO_3	0.1	6.66 ± 0.01	7.31	Pecoraro et al. [70]
pH	298.15	$NaClO_4$	0.1	6.41 ± 0.02	7.07	Shanbhag and Choppin [59]

Table 3 (continued)

D. $HADP^{2-}(aq) \rightleftharpoons H^{+}(aq) + ADP^{3-}(aq)$ (pK_{1ADP})

Method	T (K)	Medium	Ionic strength ($mol\ kg^{-1}$)	ΔH ($kJ\ mol^{-1}$)	ΔH°	Reference
Calorimetry	298.15	$(CH_3)_4NOH$	0.0556	−5.4 ±0.4	−6.9	Christensen and Izatt [60]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−5.7 ±1.3 *	−5.7	Phillips et al. [41,42]
pH	298.15	KNO_3	0.1	+5.0 ±0.4	+3.1†	Taqui Khan and Martell [53]
Calorimetry	298.15	$NaClO_4$	0.1	−2.35±0.1	−4.3	Shanbhag and Choppin [59]

E. $H_2ADP^{-}(aq) \rightleftharpoons H^{+}(aq) + HADP^{2-}(aq)$ (pK_{2ADP})

Method	T (K)	Medium	Ionic strength ($mol\ kg^{-1}$)	pK	$pK^{\circ}_{298.15}$	Reference
pH	298.15	NaCl	0.15	3.95	4.45	Alberty et al. [46]
pH	298.15	NaCl	0.1	3.9	4.34	Bock et al. [47]
pH	293.15	KCl	0.1	3.99±0.03	4.37	Martell and Schwarzenbach [48]
pH	298.15	KCl	0.1	4.21	4.65†	Weitzel and Spehr [64]
pH	298.15	$(CH_3)_4NBr$	0.0	4.20 *	4.20	Izatt and Christensen [40]
pH	298.15	KNO_3	0.1	3.93	4.37	Taqui Khan and Martell [51]
pH	298.15	KNO_3	0.1	3.93±0.01	4.37	Taqui Khan and Martell [53]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	3.87±0.06	≈ 4.46	Tate [58]
pH	298.15	$NaClO_4$	0.1	3.95	4.39	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength ($mol\ kg^{-1}$)	ΔH ($kJ\ mol^{-1}$)	ΔH°	Reference
Calorimetry	298.15	$(CH_3)_4NOH$	0.028	17.2 ±0.4	16.4	Christensen and Izatt [60]
pH	298.15	KNO_3	0.1	20.1 ±0.4	18.8	Taqui Khan and Martell [53]
Calorimetry	298.15	$NaClO_4$	0.1	18.75±0.1	17.5	Shanbhag and Choppin [59]

F. $MgADP^{-}(aq) \rightleftharpoons Mg^{2+}(aq) + ADP^{3-}(aq)$ (pK_{MgADP})

Method	T (K)	Medium	Ionic strength ($mol\ kg^{-1}$)	pK	$pK^{\circ}_{298.15}$	Reference
pH	293.15	KCl	0.1	3.11±0.05	4.47	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	3.00±0.04	4.64	Smith and Alberty [62]
Resin	296.15	NaCl	0.1	3.03	4.36	Nanninga [63]
Resin	296.15	NaCl	0.1	3.15	4.48	Walaas [65]
pH	298.15	KCl	0.1	3.23	4.54	Weitzel and Spehr [64]
Spectrophotometry	298.15	tributyl-ethylammonium bromide	0.11	3.34	4.70	Burton [71]
pH	?	$(CH_3)_4NCl$	0.05	3.34	≈ 4.37	Hotta et al. [72]
Spectrophotometry	303.15	$(C_2H_5)_4NBr$	0.1	3.61	4.88	O'Sullivan and Perrin [69]
pH	298.15	KNO_3	0.1	3.17±0.01	4.48	Taqui Khan and Martell [51]
Spectrophotometry	298.15	*N*-ethylmorpholine	0.1	3.62	4.93	Morrison et al. [73]
Fluorimetry	298.15	Tris	0.1	3.54	4.85	Watanabe et al. [74]
		triethanolamine	0.1	3.78	5.09	
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	3.40±0.10	4.71	Phillips et al. [42]
			0.0	4.27±0.10 *	4.27	
pH	298.15	KNO_3	0.10	3.17±0.02	4.48	Taqui Khan and Martell [53]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	3.69	5.29	Belaich and Sari [66]

Table 3 (continued)

F. $MgADP^{-}(aq) \rightleftharpoons Mg^{2+}(aq) + ADP^{3-}(aq)$ (pK_{MgADP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^{\circ}_{298.15}$	Reference
pH	288.15	KNO_3	0.1	3.21	4.62	Frey and Stuehr [54]
Regression	288.15	$(n\text{-}C_4H_9)_4NCl$	0.46	3.12±0.02	5.26	De Weer and Lowe [67]
Specific-ion electrode	298.15	triethanolamine	?	4.08±0.1	≈ 5.39 †	Adolfsen and Moudrianakis [75]
NMR	303.15	KNO_3	0.1	5.46±0.06	6.73 †	Pecoraro et al. [70]
pH	298.15	$NaClO_4$	0.1	3.28	4.59	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1}	ΔH (kJ mol^{-1})	ΔH°	Reference
Spectro-photometry	298.15	tributyl-ethyl-ammonium bromide	0.11	−24.7	−28.6 †	Burton [71]
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−18.0±0.3 *	−18.0	Phillips et al. [42]
pH	298.15	KNO_3	0.1	−15.1±0.8	−18.9	Taqui Khan and Martell [53]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	−13.2	−17.9	Belaich and Sari [66]
Calorimetry	298.15	$NaClO_4$	0.1	−17.5±0.1	−21.3	Shanbhag and Choppin [59]

G. $MgHADP(aq) \rightleftharpoons Mg^{2+}(aq) + HADP^{2-}(aq)$ (pK_{MgHADP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^{\circ}_{298.15}$	Reference
pH	293.15	KCl	0.1	1.5± 0.2	2.41	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	1.45±0.06	2.54	Smith and Alberty [62]
pH	298.15	KCl	0.1	1.58	2.46	Weitzel and Spehr [64]
pH	298.15	KNO_3	0.1	1.64±0.02	2.52	Taqui Khan and Martell [51]
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	1.92±0.2	2.80	Phillips et al. [42]
			0.0	2.45±0.2 *	2.45	
pH	298.15	KNO_3	0.10	1.64±0.02	2.52	Taqui Khan and Martell [53]
pH	288.15	KNO_3	0.1	1.55	2.49	Frey and Stuehr [54]
NMR	303.15	KNO_3	0.1	2.94±0.14	3.79 †	Pecoraro et al. [70]
pH	298.15	$NaClO_4$	0.10	1.90	2.78	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH°	Reference
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−3.8±5.9 *	−3.8	Phillips et al. [42]
pH	298.15	KNO_3	0.1	−16.3±0.8	−18.8	Taqui Khan and Martell [53]
Calorimetry	298.15	$NaClO_4$	0.1	−12.4±0.2	−14.9	Shanbhag and Choppin [59]

H. $HATP^{3-}(aq) \rightleftharpoons H^{+}(aq) + ATP^{4-}(aq)$ (pK_{1ATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^{\circ}_{298.15}$	Reference
pH	298.15	NaCl	0.15	6.48	7.48	Alberty et al. [46]
pH	298.15	$(C_2H_5)_4NBr$	0.15	6.98±0.02	7.98	Melchior [68]
pH	298.15	NaCl	0.1	6.5	7.38	Bock et al. [47]
pH	293.15	KCl	0.1	6.50±0.01	7.39	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	6.95±0.02	8.04	Smith and Alberty [62]
pH	298.15	KCl	0.1	6.73	7.61	Weitzel and Spehr [63]
pH	293.15	KCl	0.1	6.50	7.39	Handschin and Brintzinger [76]
pH	298.15	KNO_3	0.1	6.53	7.41	Taqui Khan and Martell [77]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	7.04±0.04	7.92	Phillips et al. [41,42]
pH	303.15	$(C_2H_5)_4NBr$	0.1	6.97±0.02	7.84	O'Sullivan and Perrin [69,78]

Table 3 (continued)

H. $HATP^{3-}(aq) \rightleftharpoons H^{+}(aq) + ATP^{4-}(aq)$ (pK_{1ATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^{\circ}_{298.15}$	Reference
Spectrophotometry	305.15	none	0.3	6.7	7.92	Khalil and Brown [79]
		KNO_3	0.6	7.0	$8.46^{\dagger}$	
pH	293.15	$NaClO_4$	0.1	6.47±0.02	7.36	Schneider et al. [80]
pH	303.15	$(CH_3)_4NBr$	0.1	6.81±0.03	7.68	Perrin and Sharma [81]
pH	298.15	KNO_3	0.1	6.53±0.01	7.41	Taqui Khan and Martell [82]
pH	288.15	KNO_3	0.1	6.57	7.47	Frey and Stuehr [54]
pH	298.15	KNO_3	1.0	6.09±0.02	7.72	Rajan et al. [83]
pH	298.15	$NaClO_4$	0.1	6.42±0.05	7.30	Sigel [52]
pH	281.15	?	?	6.7	≈ 7.62	Jaffe and Cohn [56]
pH	298.15	NaCl	0.12	6.51±0.03	7.44	Rajan et al. [84]
pH	298.15	?	0.1	7.10	≈ 7.98	Cali et al. [85]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	6.5 ±0.2	≈ 7.73	Tate [58]
NMR	303.15	KNO_3	0.1	6.63±0.04	7.50	Pecoraro et al. [70]
pH	298.15	tetraalkyl ammonium salts	0.1	6.90±0.01	7.78	De Robertis et al. [86]
pH	298.15	$NaClO_4$	0.1	6.51±0.02	7.39	Shanbhag and Choppin [59]
		$(C_2H_5)_4NCl$	0.1	6.93	7.81	

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH°	Reference
Calorimetry	298.15	$(CH_3)_4NOH$	0.0	−5.0±0.4 *	−5.0	Christensen and Izatt [60]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−7.0±1.3 *	−7.0	Phillips et al. [41,42]
pH	298.15	KNO_3	0.1	2.1±0.4	$-0.4^{\dagger}$	Taqui Khan and Martell [82]
Calorimetry	298.15	?	0.1	0.8±0.4	$-1.7^{\dagger}$	Cali et al. [85]
pH	298.15	tetraalkyl ammonium salts	0.1	−3 ±1	−5.5	De Robertis et al. [86]
Calorimetry	298.15	$NaClO_4$	0.1	−5.1±0.1	−7.6	Shanbhag and Choppin [59]

I. $H_2ATP^{2-}(aq) \rightleftharpoons H^{+}(aq) + HATP^{3-}(aq)$ (pK_{2ATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^{\circ}_{298.15}$	Reference
pH	298.15	NaCl	0.15	4.00	4.75	Alberty et al. [46]
pH	298.15	NaCl	0.1	4.1	4.76	Bock et al. [47]
pH	293.15	KCl	0.1	4.05±0.01	4.66	Martell and Schwarzenbach [48]
pH	298.15	KCl	0.1	4.26	4.92	Weitzel and Spehr [64]
pH	293.15	KCl	0.1	3.95	4.56	Handschin and Brintzinger [76]
pH	298.15	KNO_3	0.1	4.06	4.72	Taqui Khan and Martell [77]
pH	303.15	$(C_2H_5)_4NBr$	0.1	3.93±0.02	4.64	O'Sullivan and Perrin [69,78]
Spectrophotometry	305.15	none	0.3	4.65	$5.64^{\dagger}$	Khalil and Brown [79]
		KNO_3	0.6	4.55	$5.72^{\dagger}$	
pH	293.15	KCl	0.1	4.10±0.03	4.71	Schneider et al. [80]
pH	303.15	$(CH_3)_4NBr$	0.1	3.83±0.03	4.54	Perrin and Sharma [81]
pH	298.15	KNO_3	0.1	4.06±0.01	4.72	Taqui Khan and Martell [82]
pH	288.15	KNO_3	0.1	4.18	4.74	Frey and Stuehr [54]
pH	298.15	KNO_3	1.0	3.97±0.01	$5.20^{\dagger}$	Rajan et al. [83]
pH	298.15	NaCl	0.12	4.02±0.06	4.72	Rajan et al. [84]

Table 3 (continued)

I. $H_2ATP^{2-}(aq) \rightleftharpoons H^+(aq) + HATP^{3-}(aq)$ (pK_{2ATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	298.15	?	0.1	4.03	4.69	Cali et al. [85]
Electrophoresis	≈ 295.7	oxalate + buffer(s)	0.1–0.5	3.52 ± 0.04	≈ 4.42 †	Tate [58]
pH	298.15	tetraalkyl ammonium salts	0.1	3.97 ± 0.03	4.63	De Robertis et al. [86]
pH	298.15	$NaClO_4$	0.1	4.03	4.69	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH° (kJ mol^{-1})	Reference
Calorimetry	298.15	$(CH_3)_4NOH$	0.0	15.5 ± 0.4 *	15.5	Christensen and Izatt [60]
pH	298.15	KNO_3	0.1	17.2 ± 0.4	15.3	Taqui Khan and Martell [82]
Calorimetry	298.15	?	0.1	15.1 ± 1.3	13.2	Cali et al. [85]
pH	298.15	tetraalkyl ammonium salts	0.1	16 ± 3	14.1	De Robertis et al. [86]
Calorimetry	298.15	$NaClO_4$	0.1	18.4 ± 0.1	16.5	Shanbhag and Choppin [59]

J. $MgATP^{2-}(aq) \rightleftharpoons Mg^{2+}(aq) + ATP^{4-}(aq)$ (pK_{MgATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	293.15	KCl	0.1	4.00 ± 0.04	5.81	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	3.47 ± 0.04	5.66	Smith and Alberty [62]
Resin	296.15	NaCl	0.10	3.61	5.38	Nanninga [63]
Resin	296.15	NaCl	0.1	4.04	5.81	Walaas [65]
pH	298.15	KCl	0.1	4.04	5.79	Weitzel and Spehr [64]
Spectrophotometry	298.15	tributyl-ethyl-ammonium bromide	0.11	4.58	6.39	Burton [71]
Kinetic data	303.15	glycine + creatine	≈ 0.1	4.95	6.65	Noda et al. [87]
pH	?	$(CH_3)_4NCl$	0.05	3.90	≈ 5.27 †	Hotta et al. [72]
pH Resin	298.15	$(C_2H_5)_4NBr$	0.1	4.43 ± 0.03	6.18	Nanninga [88]
pH; spectrophotometry	303.15	$(C_2H_5)_4NBr$	0.1	5.02 ± 0.06	6.72	O'Sullivan and Perrin [78]
		Tris		4.30 ± 0.04	6.00	
		triethanolamine		4.89 ± 0.03	6.59	
		N-ethylymorpholine		4.93 ± 0.03	6.63	
Interferometry	296.15	Tris	0.1	3.88 ± 0.1	5.65	Asai and Morales [89]
pH	293.15	KCl	0.1	3.84	5.65	Handschin and Brintzinger [76]
pH	298.15	KCl	0.1	4.22 ± 0.01	5.97	Taqui Khan and Martell [77]
Fluorimetry	298.15	Tris	0.1	3.63 ± 0.10	5.38	Watanabe et al. [74]
		triethanolamine	0.1	3.85 ± 0.05	5.60	
pH; spectrophotometry	303.15	$(C_2H_5)_4NBr$	0.1	4.94	6.64	O'Sullivan and Perrin [69]
		Tris		4.30 ± 0.04	6.00	
		triethanolamine		4.89 ± 0.03	6.59	
		N-ethylmorpholine		4.90 ± 0.04	6.60	
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	4.55 ± 0.10	6.30	Phillips et al. [42]
			0.0	5.83 ± 0.10 *	5.83	
pH	298.15	KNO_3	0.1	4.22 ± 0.02	5.97	Taqui Khan and Martell [82]
pH	303.15	$(CH_3)_4NCl$	0.3	4.85	7.26 †	Noat et al. [90]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	4.69	6.83	Belaich and Sari [66]

Table 3 (continued)

J. $MgATP^{2-}(aq) \rightleftharpoons Mg^{2+}(aq) + ATP^{4-}(aq)$ (pK_{MgATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	288.15	KNO_3	0.1	4.05	5.91	Frey and Stuehr [54]
regression	288.15	$(n\text{-}C_4H_9)_4NCl$	0.46	4.11 ± 0.03	6.95 †	De Weer and Lowe [67]
Specific-ion electrode	298.15	?	≈ 0.005	6.06	6.58	Mohan and Rechnitz [91]
pH	298.15	KNO_3	1.0	3.22 ± 0.02	6.49	Rajan et al. [83]
Specific-ion electrode	298.15	triethanolamine	?	5.15 ± 0.07	≈ 6.90 †	Adolfsen and Moudrianakis [75]
pH	281.15	?	?	5.3	≈ 7.24 †	Jaffe and Cohn [56]
pH	298.15	$NaClO_4$	0.1	4.24 ± 0.02	5.99	Mitchell and Sigel [92]
pH	298.15	NaCl	0.12	4.01 ± 0.06	5.87	Rajan et al. [84]
Spectrophotometry	298.15	?	?	4.72 ± 0.04	≈ 6.47	Bishop et al. [93]
Calorimetry	310.15	$(CH_3)_4NCl$	0.2	4.73 ± 0.03	6.81	Sari et al. [94]
NMR	303.15	KNO_3	0.1	4.72 ± 0.05	6.42	Pecoraro et al. [70]
pH	298.15	tetraalkyl ammonium salts	0.1	4.54 ± 0.06	6.29	De Robertis et al. [86]
pH	298.15	$NaClO_4$	0.1	4.03	5.78	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH°	Reference
Resin	298.15	NaCl	0.10	−17.2	−22.3	Nanninga [63]
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−21.3 ± 1.3 *	−21.3	Phillips et al. [42]
pH	298.15	KNO_3	0.1	−10.9 ± 0.4	−15.9 †	Taqui Khan and Martell [82]
Calorimetry	303.15	$(CH_3)_4NCl$	0.2	−18.7	−25.5	Belaich and Sari [66]
pH	298.15	tetraalkyl ammonium salts	0.1	−15 ± 3	−20.1	De Robertis et al. [86]
Calorimetry	310.15	$(CH_3)_4NCl$	0.2	−18.3 ± 0.1	−25.7	Sari et al. [94]
Calorimetry	298.15	$NaClO_4$	0.1	−18.1 ± 0.1	−23.2	Shanbhag and Choppin [59]

K. $MgHATP^{-}(aq) \rightleftharpoons Mg^{2+}(aq) + HATP^{3-}(aq)$ (pK_{MgHATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
pH	293.15	KCl	0.1	2.0 ± 0.1	3.35	Martell and Schwarzenbach [48]
pH	298.15	$(n\text{-}C_3H_7)_4NBr$	0.2	1.49 ± 0.09	3.13 †	Smith and Alberty [62]
pH	298.15	KCl	0.1	2.16	3.47	Weitzel and Spehr [64]
pH	293.15	KCl	0.1	2.09	3.44	Handschin and Brintzinger [76]
pH	298.15	KNO_3	0.1	2.24 ± 0.01	3.55	Taqui Khan and Martell [77]
pH	303.15	$(C_2H_5)_4NBr$	0.1	2.85	4.13	O'Sullivan and Perrin [69]
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.1	2.72 ± 0.12	4.03	Phillips et al. [42]
			0.0	3.59 ± 0.12 *	3.59	
pH	298.15	KNO_3	0.1	2.24 ± 0.02	3.55	Taqui Khan and Martell [82]
pH	288.15	KNO_3	0.1	2.18	3.58	Frey and Stuehr [54]
NMR	303.15	KNO_3	0.1	2.79 ± 0.15	4.07	Pecoraro et al. [70]
pH	298.15	$NaClO_4$	0.1	2.12	3.43	Shanbhag and Choppin [59]

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH°	Reference
Resin	298.15	$(n\text{-}C_3H_7)_4NBr$	0.0	−9.2 ± 5.4 *	−9.2	Phillips et al. [42]
pH	298.15	KNO_3	0.1	−14.2 ± 0.4	−18.0	Taqui Khan and Martell [82]
Calorimetry	298.15	$NaClO_4$	0.1	−19.7 ± 0.2	−23.5	Shanbhag and Choppin [59]

Table 3 (continued)

L. $Mg_2ATP(aq) \rightleftharpoons Mg^{2+}(aq) + MgATP^{2-}(aq)$ (pK_{Mg2ATP})

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	pK	p$K^\circ_{298.15}$	Reference
Kinetic data	303.15	?	≈ 0.3	1.60	2.81	Noat et al. [95]
Specific-ion electrode	298.15	–	0.005–0.010	2.61 ± 0.05	2.92	Mohan and Rechnitz [91]
NMR	298.15	?	?	1.52 ± 0.05	2.40	Bishop et al. [93]
Calorimetry	310.15	$(CH_3)_4NCl$	0.2	1.69	2.73	Sari et al. [94]

Method	T (K)	Medium	Ionic strength (mol kg^{-1})	ΔH (kJ mol^{-1})	ΔH° (kJ mol^{-1})	Reference
Calorimetry	310.15	$(CH_3)_4NCl$	0.2	−7.2 ± 0.5	−10.9 ± 1.5	Sari et al. [94]

magnesium-ion binding constants and our own equilibrium data [7] on the adenylate kinase reaction in which the ionic strength was purposefully varied. This value is very close to the value of 1.6 kg$^{1/2}$ mol$^{-1/2}$ obtained from fitting data for typical uni–uni and uni–bivalent electrolytes and it will be used in all subsequent calculations. While a higher value for B would give a better representation of the proton and magnesium-ion binding constants, it clearly does not work for the data on the adenylate kinase reaction. Thus, the value of 1.5 kg$^{1/2}$ mol$^{-1/2}$ obtained from the empirical fit to our equilibrium data, was used for B in all our thermodynamic calculations.

This value of B was then used to adjust the results obtained in the various investigations to the standard state. The results so obtained are given in the next to last column in Table 3. Adjustments of the equilibrium data to 298.15 K were also made when needed with the final selected values of the enthalpy changes for the respective reactions (see Table 1). In several cases, the investigators failed to report the temperature and/or the ionic strength at which they made their measurements. In such cases, we have assumed that the temperature was 298.15 K and that the ionic strength was 0.1 mol kg^{-1}. Note that while the adjustments to the standard state are large, the equilibrium constants and enthalpies of reaction that are obtained will later be adjusted back to experimentally accessible ionic strengths (typically 0.1 to 0.5 mol kg^{-1}) in treating the adenylate kinase equilibrium data. Subsequent use of the final set of proton and magnesium-ion binding constants in obtaining a value of B from our data on the adenylate kinase reaction again led to a value of 1.5 kg$^{1/2}$ mol$^{-1/2}$ for this parameter. A minor point to be noted is that the molality standard state is being used in our calculations while most of the proton and metal-ion binding constants were based on the concentration standard state. For the reactions in Table 3 the adjustment between these two standard states is 0.0013 at 298.15 K and 0.0025 at 310.15 K. This small correction is an order of magnitude smaller than the uncertainties in the proton and metal-ion binding constants and it will be neglected.

The much more serious issue here is the accuracy of the proton and metal-ion binding constants. Most of them have been determined with either a glass electrode to measure the pH or by spectrophotometric methods or NMR. Unfortunately, there have been no studies that have used either conductivity or electrochemical cells without liquid junctions to determine thermodynamic parameters for these various reactions. These methods when carefully applied to specific systems are generally preferable [44]. Also of concern here is the matter of the activity coefficients to be used in the adjustment of the data to the standard state and the possible complication of binding of other metal ions such as sodium and potassium. In spite of these complications and uncertainties, we have made a somewhat arbitrary selection of binding constants which is essentially based upon an averaging of the data in Table 3. These averages are given in Table 1 with the uncertainties being equal to two standard deviations. Examination of Table 1 indicate some interesting trends in the entropy changes for the various reactions. For example, the quantities $\{\Delta S^{\circ}_{1\mathrm{AMP}} - \Delta S^{\circ}_{2\mathrm{AMP}}\}$, $\{\Delta S^{\circ}_{1\mathrm{ADP}} - \Delta S^{\circ}_{2\mathrm{ADP}}\}$, and $\{\Delta S^{\circ}_{1\mathrm{ATP}} - \Delta S^{\circ}_{2\mathrm{ATP}}\}$ are respectively equal to -131, -132, and -127 J mol^{-1} K^{-1}. The quantities $\{\Delta S^{\circ}_{\mathrm{MgHADP}} - \Delta S^{\circ}_{\mathrm{MgADP}}\}$ and $\{\Delta S^{\circ}_{\mathrm{MgHATP}} - \Delta S^{\circ}_{\mathrm{MgATP}}\}$ are respectively equal to 63 and 69 J mol^{-1} K^{-1}. Finally, $\Delta S^{\circ}_{\mathrm{MgAMP}}$, $\Delta S^{\circ}_{\mathrm{MgHADP}}$, and $\Delta S^{\circ}_{\mathrm{Mg2ATP}}$ are equal to -92, -90, and -89 J mol^{-1} K^{-1}, respectively. These findings are consistent with the often made observation that entropies and entropy changes correlate well with structural and other chemical features.

The development of the model and the selection of the auxiliary data needed for it is now complete. The experimental equilibrium and calorimetric data for the adenylate kinase reaction are dealt with in Part II of this paper [7].

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Notation

a	activity
A_m	Debye-Hückel constant
B	parameter in the Debye–Hückel equation
C_p	heat capacity
f	fraction of a substance in a specified form
G	Gibbs energy
H	enthalpy
I	ionic strength
K	equilibrium constant
m	molality
n	amount of substance
p	pressure
pK	$-\log K$
pX	$-\log a(X)$, where X is any solute species
q	heat
R	gas constant
S	entropy
T	thermodynamic temperature
z	charge number of an ion

Greek

γ	activity coefficient
Γ	activity coefficient ratio
θ	reference temperature (298.15 K)
λ, λ_{ij}	pair-wise interaction parameter
ν	stoichiometric number
ξ	extent of reaction
σ	defined quantity (see eq. 39)
Σ	summation sign; or denotes total amounts of substances

Superscripts

ex	excess quantity
°	standard quantity
*	property of a pure substance
′	apparent quantity

Subscripts

i, j	denote species i and j
±	mean ionic quantity
ref	abbreviation of reference

Suffixes

ao	aqueous, standard state of the indicated species
aq	aqueous, concentration not specified

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