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

## II. Experimental data

Yadu B. Tewari and Robert N. Goldberg

*Chemical Thermodynamics Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA*

and

Javher V. Advani

*U. S. Food and Drug Administration, 1600 Fisher's Lane, Rockville, Maryland 20857, USA*

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High-pressure liquid-chromatography and microcalorimetry have been used to determine equilibrium constants and enthalpies of reaction for the disproportionation reaction of adenosine 5′-diphosphate (ADP) to adenosine 5′-triphosphate (ATP) and adenosine 5′-monophosphate (AMP). Adenylate kinase was used to catalyze this reaction. The measurements were carried out over the temperature range 286 to 311 K, at ionic strengths varying from 0.06 to 0.33 mol kg$^{-1}$, over the pH range 6.04 to 8.87, and over the pMg range 2.22 to 7.16, where pMg $= -\log a(Mg^{2+})$. The equilibrium model developed by Goldberg and Tewari (see the previous paper in this issue) was used for the analysis of the measurements. Thus, for the reference reaction: 2 $ADP^{3-}$(ao) $\rightleftharpoons$ $AMP^{2-}$(ao) + $ATP^{4-}$(ao), $K^\circ = 0.225 \pm 0.010$, $\Delta G^\circ = 3.70 \pm 0.11$ kJ mol$^{-1}$, $\Delta H^\circ = -1.5 \pm 1.5$ kJ mol$^{-1}$, $\Delta S^\circ = -17 \pm 5$ J mol$^{-1}$ K$^{-1}$, and $\Delta C_p^\circ \approx -46$ J mol$^{-1}$ K$^{-1}$ at 298.15 K and 0.1 MPa. These results and the thermodynamic parameters for the auxiliary equilibria in solution have been used to model the thermodynamics of the disproportionation reaction over a wide range of temperature, pH, ionic strength, and magnesium ion molality. Under approximately physiological conditions (311.15 K, pH 6.94, $[Mg^{2+}] = 1.35 \times 10^{-3}$ mol kg$^{-1}$, and $I = 0.23$ mol kg$^{-1}$) the apparent equilibrium constant ($K'_A = m(\Sigma AMP)m(\Sigma ATP)/[m(\Sigma ADP)]^2$) for the overall disproportionation reaction is equal to $0.93 \pm 0.02$. Thermodynamic data on the disproportionation reaction and literature values for this apparent equilibrium constant in human red blood cells are used to calculate a molality of $1.94 \times 10^{-4}$ mol kg$^{-1}$ for free magnesium ion in human red blood cells. The results are also discussed in relation to thermochemical cycles and compared with data on the hydrolysis of the guanosine phosphates.

## 1. Introduction

In the preceding paper [1], an equilibrium model was developed to describe the thermodynamics of the disproportionation reaction of adenosine 5′-diphosphate (ADP) to adenosine 5′-triphosphate (ATP) and adenosine 5′-monophosphate (AMP) [1]. As that model was being developed, the data from the literature [2–15] were also collected and then

[1] The nomenclature, symbols, and abbreviations used herein are the same as those in the preceding paper [1].

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examined with an algorithm based upon that model. These data, for the most part, had not previously been subjected to this type of analysis. The calculations showed large discrepancies between the results of different investigations. In particular, there were only two investigations [4,15] in which equilibrium data had been determined as a function of temperature. Indeed, these two investigations differed with respect to the direction with which the equilibrium constant varied with temperature. Also, values of the equilibrium constant for the reference reaction:

$$2\,ADP^{3-}(ao) \rightleftharpoons AMP^{2-}(ao) + ATP^{4-}(ao) \quad (A)$$

which were calculated from the original data varied over the range 0.2 to 2.0 at 298.15 K and 0.1 MPa. These discrepancies motivated this experimental investigation in which we used high-pressure liquid-chromatography (HPLC) for the determination of apparent equilibrium constants. An enthalpy change was also calculated from the temperature dependence of the equilibrium data and examined in relationship to the more accurate enthalpy change determined by direct calorimetry. This method allows for a good check on the accuracy of the overall set of measurements. We also performed experiments where pH, ionic strength, magnesium ion molality, and temperature were varied systematically. These results can then be compared with the predictions of the equilibrium model developed in the preceding paper [1]. In this way, an overall picture of the thermodynamics of the disproportionation reaction of ADP can be obtained.

## 2. Experimental

The monosodium salt of AMP, the monopotassium salt of ADP, and the disodium salt of ATP, the adenylate kinase, and the TRIS buffer were obtained from Sigma Chemical Company [2], St. Louis, MO. Magnesium chloride hexahydrate was from Fisher Scientific Company. The potassium dihydrogen phosphate and disodium hydrogen phosphate used to prepare the standard "physiological" buffer and also in a few equilibrium measurements were, respectively, Standard Reference Materials 186-I-d and 186-II-d from the National Institute of Standards and Technology. The nucleotides and the adenylate kinase were stored in desiccators at $-25\,°C$. The moisture contents of the nucleotides and of the magnesium chloride in mass percent, as determined by Karl Fischer titration, were: AMP, $15.9 \pm 0.3$; ADP, $7.0 \pm 0.4$; ATP, $10.7 \pm 0.5$; and magnesium chloride, $54.2 \pm 1.5$. The moisture content of the magnesium chloride was also determined by drying it in an oven at $140\,°C$ to constant weight. The result of this analysis was a moisture content of $53.5 \pm 0.9$ mass percent in good agreement with the result obtained by the Karl Fischer method. However, the result obtained by drying to constant weight was judged to be more reliable than the result from the Karl Fischer determination and it was used in calculating the molalities of $MgCl_2$ in the various solutions. All of the nucleotides were assayed for impurities with the chromatographic procedures described below. From these assays it was found that the ATP contained $0.50 \pm 0.02$ mole percent ADP and that the ADP contained $0.39 \pm 0.02$ mole percent AMP. The AMP was found to be chromatographically pure. The results of these assays and of the moisture determinations were applied as corrections to all of the measurements performed as a part of this study. The adenylate kinase as received from the vendor was in lyophilized form and had been obtained from rabbit muscle. The chromatographic analysis showed no evidence of any side reactions accompanying the disproportionation reaction. The average recovery of the nucleotides in the equilibrium measurements was $100.5 \pm 0.5$ percent. Here the recovery is defined as the quantity:

$$100 \times [\text{total nucleotide determined chromatographically}] / [\text{total nucleotide weighed into solution}].$$

This recovery is consistent with the absence of side reactions.

[2] Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

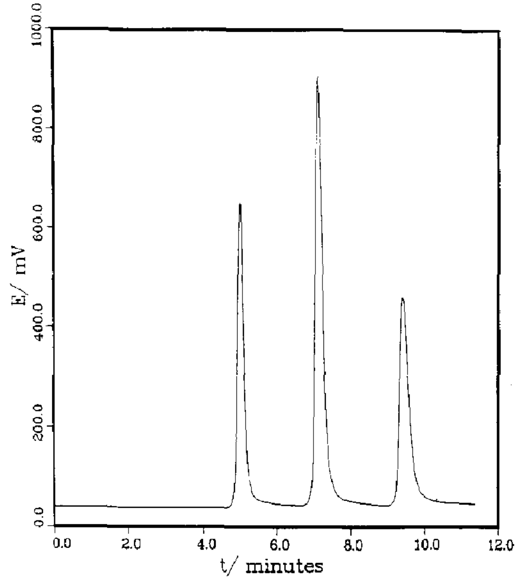


Fig. 1. Chromatogram showing the separation and quantitative determination of AMP, ADP, and ATP. The chromatographic analysis was done with a Hewlett-Packard 1090 HPLC with a Serva DEAE Si 100 anion exchange column. The mobile phase was a gradient of (A) 0.01 *M* phosphate at pH 2.9 and (B) 0.25 *M* phosphate at pH 7.3. At time zero the gradient was 85% (A) and 15% (B). At 25.0 minutes the gradient was 100% (B). The flow rate was 1.6 $cm^3$ $min^{-1}$. A diode-array detector set at 254 nm was used for detection. The retention times for AMP, ADP, and ATP are 5.0, 7.1, and 9.4 minutes, respectively.

Chromatographic analyses were done with a Hewlett-Packard 1090 HPLC with a Serva DEAE Si 100 anion exchange column. The mobile phase was a gradient of (A) 0.01 mol $dm^{-3}$ phosphate at pH 2.9 and (B) 0.25 mol $dm^{-3}$ phosphate at pH 7.3. At time zero the gradient was 85% (A) and 15% (B). At 25.0 minutes the gradient was 100% (B). The flow rate of the mobile phase was 1.6 $cm^3$ $min^{-1}$. A diode-array detector set at 254 nm was used for measurement of the amounts of nucleotides. All injections were done with a fixed-loop device. Typical retention times were 5.0, 7.1, and 9.4 minutes for the AMP, ADP, and ATP, respectively (see Fig. 1). The separation of the peaks for the nucleotides was complete in all cases. Response factors were determined on a daily basis and used to calculate the molalities of the nucleotides in solution.

Chromatographic data were recorded and analyzed with a Dionex AI-450 computer controlled data acquisition system. This system makes use of an analog to digital conversion of the signal from, in this case, the diode-array detector. The digital data are recorded on hard disk and accurately integrated. These peaks can also be examined more closely at any later time to determine whether there are any difficulties with the baseline and shoulders.

Solutions of the substrates in buffer containing the adenylate kinase and a known amount of magnesium chloride were allowed to equilibrate with gentle stirring in a thermostatted water bath. Equilibrium was approached from two directions: starting with ADP (the forward direction) and starting with a mixture containing approximately equal amounts of AMP and ATP (the reverse direction). It was found that chemical equilibrium, as evidenced by the agreement of equilibrium ratios determined from both the forward and reverse directions, was attained within two days in all cases and within one day in most cases.

The calorimeters are of the heat-conduction type and have calibration constants varying from 17 to 22 W $V^{-1}$. The sensitivity (units of V $W^{-1}$) is the inverse of the calibration constant. Each calorimeter contains two thermopiles which are solid-state bismuth selenide — bismuth telluride — bismuth antimonide thermoelectric modules manufactured by Cambion Corporation. The two thermopiles are connected in series and are situated in an aluminum block which is contained in an air thermostat, the temperature of which is controlled by means of a Wheatstone bridge circuit and a controller which utilizes both proportional and integral modes of action. The sample vessels, which are fabricated from high-density polyethylene, contain two compartments holding approximately 0.55 and 0.45 $cm^3$ of solution, respectively. The sample vessels are loaded into a copper container which is kept glued in place between the thermopile elements in a sandwich type arrangement. The vessels and their contents are allowed to equilibrate for at least one hour before the solutions in the vessel are mixed. The calibration of the calorimeters is done electrically with a calibrated voltmeter, standard resistor, and time-interval counter. The inaccuracy and imprecision of the measurements ($\approx 0.2\%$ for a total heat of 300 mJ) have been determined with chemical reactions which produce well known amounts of heat. Complete descriptions of the calorimeters and their performance characteristics are given in refs. [16] and [17].

Measurements of reaction heat were performed by mixing, in the calorimeter, a substrate solution and an enzyme solution. The substrate solution was prepared by dissolving a known amount of the ADP in a TRIS buffer solution containing a known amount of magnesium chloride. The enzyme solution was prepared by adding the same buffer solution to the lyophilized adenylate kinase. The extent of reaction was determined by analyses of the reaction mixtures immediately following completion of the heat measurements. Typically, the calorimetric measurements lasted for about one hour after which the contents of the reaction vessels were analyzed with the chromatograph. These chromatographic analyses yielded apparent equilibrium constants in agreement with the results of the equilibrium experiments. This indicated that reaction had proceeded as far as possible. The calorimetric measurements also showed that, one hour after the mixing of the enzyme and substrate solutions, there was no heat being produced. This is consistent with the absence of any additional reactions. The "blank" heats accompanying the mixing of the substrate solution and of the enzyme solution with the buffer were, respectively, $-(0.63 \pm 0.41)$ mJ and $(0.27 \pm 0.84)$ mJ. Thus, a combined correction of $-(0.36 \pm 1.0)$ mJ was applied to the heats measured for the disproportionation reaction. Measured heats were in the range 1 to 4 mJ. This correction is the primary uncertainty in the determination of the enthalpy change for the disproportionation reaction.

The measurement of the pH of the final reaction mixtures in both the equilibrium and calorimetric experiments was done with a combination glass micro-electrode and an Orion Model 811 pH meter. All measurements were done at the temperature at which the disproportionation reaction occurred, either in the microcalorimeter(s) or in the thermostats used for the equilibrium measurements. Calibration was done with a standard buffer prepared from potassium dihydrogen phosphate (0.009695 mol $kg^{-1}$) and disodium hydrogen phosphate (0.03043 mol $kg^{-1}$). Intercomparisons of this "physiological" buffer against Fisher buffers certified at pH values of 7.0, 8.0, and 9.0 was also done with satisfactory agreement ($\pm 0.03$) in the pH of these solutions. A measurement of the difference in pH brought about by the disproportionation reaction yielded a result of a drop in pH equal to $0.001 \pm 0.018$. Thus, the amount of protons produced or absorbed is negligible within the experimental imprecision of this measurement.

All solutions used in both the calorimetric and equilibrium measurements were prepared gravimetrically with balances which had been checked against calibrated weights.

## 3. Results and discussion

The results of the equilibrium and calorimetric experiments are given in Tables 1 and 2, respectively. The apparent equilibrium constant:

$$K'_A = m(\Sigma\text{AMP})m(\Sigma\text{ATP})/[m(\Sigma\text{ADP})]^2 \qquad (1)$$

given in Table 1 has been determined over a wide range of experimental conditions, namely $T = 286$ to 311 K, pH = 6.04 to 8.87, $I = 0.06$ to 0.33 mol $kg^{-1}$, and pMg = 2.22 to 7.16. The imprecision with which the apparent equilibrium constants could be determined is seen to be remarkably good in many cases. However, we judge that uncertainties in the determination of response factors, moisture contents, and sample purities lead to a realistic error estimate of about $\pm 2\%$ for the apparent equilibrium constants given in Table 1. The enthalpy change ($\Delta H'_A$) given in Table 2 is equal to the measured heat, which has been corrected for blank heats (see Section 2), divided by the amount of ADP undergoing disproportionation. Included in $\Delta H'_A$ are the following enthalpy changes: $\Delta H^\circ$ for the reference reaction (A); enthalpies of proton and magnesium ion binding; the enthalpy differences for the conversion of reactants and products from the standard state to the actual experimental conditions; and the enthalpy of protonation of the Tris buffer. The analysis of these results will be done in terms of the model developed in the preceding paper [1]. The proton and magnesium ion binding constants given in Table 1 of ref. [1] will be used in all subsequent calculations. The first aim will be the extraction of a set of thermodynamic parameters ($K^\circ$, $\Delta G^\circ$, $\Delta H^\circ$, $\Delta S^\circ$, and $\Delta C_p^\circ$) for the reference reaction (A) from experiments which were performed under conditions where possible errors in the proton and magnesium-ion binding corrections have the least effect. After this has been done, the model will be used to predict the variation of the equi-

Table 1

Equilibrium data for the disproportionation of ADP to AMP and ATP. The equilibrium constant ($K'_A$) is equal to the quantity $\{m(\Sigma AMP)m(\Sigma ATP)/[m(\Sigma ADP)]^2\}$. Each apparent equilibrium constant is the average of four to six measurements. Uncertainties are statistical 95% confidence limits. Experiments 1–38 were done with TRIS buffer while experiments 39 and 40 were done with phosphate buffer. The concentrations of adenylate kinase was $\approx 0.2$ g kg$^{-1}$. The ionic strength and pMg are calculated quantities

| Expt. no. | Direction of reaction | $K'_A$ | T (K) | pH | Nucleotide (mol kg$^{-1}$) | $MgCl_2$ | Buffer | KCl | $I$ | pMg |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | forward | 0.343 ± 0.009 | 298.15 | 8.31 | 0.008247 | 0.0005101 | 0.04982 | 0.0 | 0.072 | 6.72 |
| 2 | reverse | 0.346 ± 0.006 | 298.15 | 8.54 | 0.008794 | 0.0005099 | 0.04982 | 0.0 | 0.081 | 6.71 |
| 3 | forward | 0.355 ± 0.018 | 286.05 | 8.68 | 0.008247 | 0.0005101 | 0.04979 | 0.0 | 0.072 | 6.59 |
| 4 | reverse | 0.356 ± 0.017 | 286.05 | 8.85 | 0.008794 | 0.0005099 | 0.04979 | 0.0 | 0.080 | 6.59 |
| 5 | forward | 0.352 ± 0.003 | 292.15 | 8.46 | 0.008247 | 0.0005101 | 0.04982 | 0.0 | 0.071 | 6.66 |
| 6 | reverse | 0.349 ± 0.003 | 292.15 | 8.67 | 0.008794 | 0.0005099 | 0.04979 | 0.0 | 0.080 | 6.65 |
| 7 | forward | 0.357 ± 0.005 | 304.15 | 8.16 | 0.008247 | 0.0005101 | 0.04982 | 0.0 | 0.072 | 6.78 |
| 8 | reverse | 0.351 ± 0.002 | 304.15 | 8.34 | 0.008794 | 0.0005099 | 0.04979 | 0.0 | 0.080 | 6.78 |
| 9 | forward | 0.351 ± 0.004 | 310.25 | 7.98 | 0.008247 | 0.0005101 | 0.04982 | 0.0 | 0.071 | 6.84 |
| 10 | reverse | 0.348 ± 0.003 | 310.25 | 8.13 | 0.008794 | 0.0005099 | 0.04979 | 0.0 | 0.078 | 6.84 |
| 11 | forward | 0.381 ± 0.004 | 298.15 | 8.34 | 0.008006 | 0.002412 | 0.04978 | 0.0 | 0.070 | 6.02 |
| 12 | reverse | 0.378 ± 0.002 | 298.15 | 8.54 | 0.008817 | 0.002410 | 0.04975 | 0.0 | 0.080 | 6.02 |
| 13 | forward | 0.861 ± 0.004 | 298.15 | 8.28 | 0.008370 | 0.02433 | 0.04932 | 0.0 | 0.061 | 4.70 |
| 14 | reverse | 0.854 ± 0.002 | 298.15 | 8.51 | 0.008506 | 0.02432 | 0.04931 | 0.0 | 0.068 | 4.69 |
| 15 | forward | 0.260 ± 0.002 | 298.15 | 8.40 | 0.008117 | 0.2372 | 0.04495 | 0.0 | 0.093 | 2.24 |
| 16 | reverse | 0.258 ± 0.004 | 298.15 | 8.56 | 0.009037 | 0.2371 | 0.04494 | 0.0 | 0.096 | 2.27 |
| 17 | forward | 0.634 ± 0.004 | 298.15 | 8.33 | 0.008131 | 0.07690 | 0.04824 | 0.0 | 0.056 | 3.12 |
| 18 | reverse | 0.603 ± 0.003 | 298.15 | 8.53 | 0.008485 | 0.07688 | 0.04823 | 0.0 | 0.062 | 3.16 |
| 19 | forward | 0.475 ± 0.002 | 298.15 | 8.30 | 0.008406 | 0.007455 | 0.04966 | 0.0 | 0.069 | 5.49 |
| 20 | reverse | 0.484 ± 0.004 | 298.15 | 8.50 | 0.008725 | 0.007453 | 0.04965 | 0.0 | 0.076 | 5.48 |
| 21 | forward | 1.302 ± 0.012 | 298.15 | 8.32 | 0.008166 | 0.03994 | 0.04900 | 0.0 | 0.057 | 4.07 |
| 22 | reverse | 1.268 ± 0.017 | 298.15 | 8.50 | 0.008631 | 0.03993 | 0.04898 | 0.0 | 0.063 | 4.13 |
| 23 | forward | 0.349 ± 0.006 | 298.15 | 8.40 | 0.007664 | 0.0001959 | 0.04983 | 0.0 | 0.071 | 7.12 |
| 24 | reverse | 0.361 ± 0.005 | 298.15 | 8.54 | 0.008526 | 0.0001957 | 0.04980 | 0.0 | 0.079 | 7.12 |
| 25 | forward | 0.408 ± 0.005 | 298.15 | 8.49 | 0.008226 | 0.0001958 | 0.04982 | 0.0491 | 0.125 | 6.92 |
| 26 | reverse | 0.412 ± 0.003 | 298.15 | 8.67 | 0.008715 | 0.0001950 | 0.04961 | 0.0489 | 0.132 | 6.93 |
| 27 | forward | 0.450 ± 0.003 | 298.15 | 8.57 | 0.008122 | 0.0001957 | 0.04982 | 0.1506 | 0.228 | 6.67 |
| 28 | reverse | 0.444 ± 0.003 | 298.15 | 8.73 | 0.008903 | 0.0001934 | 0.04924 | 0.1488 | 0.233 | 6.70 |
| 29 | forward | 0.462 ± 0.003 | 298.15 | 8.68 | 0.007159 | 0.0001958 | 0.04984 | 0.2540 | 0.329 | 6.46 |
| 30 | reverse | 0.458 ± 0.003 | 298.15 | 8.87 | 0.006955 | 0.0001922 | 0.04891 | 0.2493 | 0.326 | 6.46 |
| 31 | forward | 0.366 ± 0.002 | 298.15 | 7.79 | 0.007301 | 0.0002121 | 0.05018 | 0.0 | 0.052 | 7.13 |
| 32 | reverse | 0.364 ± 0.002 | 298.15 | 7.93 | 0.008351 | 0.0002119 | 0.05014 | 0.0 | 0.061 | 7.15 |
| 33 | forward | 0.366 ± 0.002 | 298.15 | 6.04 | 0.007318 | 0.0002121 | 0.05018 | 0.0 | 0.026 | 6.34 |
| 34 | reverse | 0.377 ± 0.003 | 298.15 | 6.67 | 0.008351 | 0.0002119 | 0.05014 | 0.0 | 0.037 | 6.84 |
| 35 | forward | 0.364 ± 0.002 | 298.15 | 6.77 | 0.007418 | 0.0001700 | 0.05017 | 0.0 | 0.034 | 6.97 |
| 36 | reverse | 0.365 ± 0.001 | 298.15 | 6.88 | 0.007988 | 0.0001699 | 0.05015 | 0.0 | 0.038 | 7.05 |
| 37 | forward | 0.365 ± 0.001 | 298.15 | 7.41 | 0.007619 | 0.0001700 | 0.05017 | 0.0 | 0.044 | 7.22 |
| 38 | reverse | 0.366 ± 0.002 | 298.15 | 7.45 | 0.008248 | 0.0001699 | 0.05014 | 0.0 | 0.048 | 7.24 |
| 39 | forward | 0.934 ± 0.001 | 311.15 | 6.83 | 0.002193 | 0.02426 | 0.00967 | 0.2303 | 0.249 | 3.51 |
| 40 | reverse | 0.928 ± 0.002 | 311.15 | 7.04 | 0.002177 | 0.02429 | 0.00951 | 0.2264 | 0.245 | 3.54 |

librium data with pH, pMg, ionic strength, and temperature. Detailed comparisons of the calculated and measured values of $K'_A$ will then be made.

Auxiliary data for the ionization of Tris and phosphate buffers were also required in these calculations. Thus for the reaction:

$$TRIS \cdot H^+(ao) \rightleftharpoons TRIS(ao) + H^+(ao) \quad (B)$$

we have used $pK° = 8.07$, $\Delta H° = 47.48$ kJ $mol^{-1}$, and $\Delta C_p° = -50$ J $mol^{-1}$ $K^{-1}$ at 298.15 K and 0.1 MPa based upon data given in refs. [18–20]. For the reaction:

$$H_2PO_4^-(ao) = H^+(ao) + HPO_4^{2-}(ao) \quad (C)$$

we have used [21,22] $pK° = 7.21$, $\Delta H° = 3.60$ kJ $mol^{-1}$, and $\Delta C_p° = -220$ J $mol^{-1}$ $K^{-1}$ at 298.15 K. In treating the experimental results of Lawson and Veech [12] information on the binding of $Mg^{2+}$ to $HPO_4^{2-}$ was also needed. We have used the data of Clarke et al. [23,24], namely $pK° = 2.70$, $\Delta H° = -12.2$ kJ $mol^{-1}$, and $\Delta C_p° = -251$ J $mol^{-1}$ $K^{-1}$, for the reaction:

$$MgHPO_4(ao) \rightleftharpoons Mg^{2+}(ao) + HPO_4^{2-}(ao) \quad (D)$$

Experiments 1, 2, and 23 to 30 in Table 1 were performed at high values of pH and pMg at 298.15 K and include variations in the ionic strength. From these results, values of $K_A° = 0.225 \pm 0.010$ for the reference reaction and of $B = 1.5 \pm 0.3$ $kg^{1/2}$ $mol^{-1/2}$ were obtained as a result of a least-squares calculation. It was found that the introduction of various values for $\lambda$ in the activity coefficient expression (eq. 32 in ref. [1]) had no effect on the results of these calculations. Hence it was not included in any subsequent calculations. The uncertainty assigned to $K°$ is twice as large as the statistical 95% uncertainty interval. This was done primarily to allow for a possible error in the adjustment to the standard state. The corresponding Gibbs energy change for the reference reaction (A) is $3.70 \pm 0.11$ kJ $mol^{-1}$. Similarly, with the equilibrium data determined as a function of temperature (experiments 1 to 10, 23, and 24) and at high pH and pMg, a value of $\Delta H_A° = -0.9 \pm 1.1$ kJ $mol^{-1}$ at 298.15 K was obtained.

The equilibrium model was also used to treat the calorimetric data in Table 2 and to obtain the calculated values of $\Delta H_A°$ given in the last column in the lower part of that table. The intermediate quantities used in this calculation (see eq. 41 in ref. [1]) are also given in the lower part of Table 2. Thus, from the calorimetric experiments the following results are obtained: $\Delta H_A° = -2.1 \pm 1.0$ kJ $mol^{-1}$ and $\Delta C_{p,A}° = -46 \pm 90$ J $mol^{-1}$ $K^{-1}$ at 298.15 K. This calculated heat capacity change must be considered to be only an approximate

Table 2

Calorimetric data for the disproportionation reaction of ADP to AMP and ATP. The enthalpy changes ($\Delta H'_A$) given in column 8 in the upper part of the table are equal to the measured heats, which have been corrected for blank heats (see Section 2), divided by the amounts of ADP undergoing disproportionation. Each result is the average of five or six replicate measurements where the total molalities of ADP, $MgCl_2$, and Tris (given in columns 5 to 7 in the upper part of the table) used in each calorimetric vessel are, respectively, very nearly equal. The concentration of adenylate kinase was $\approx 0.4$ g $kg^{-1}$ in all experiments. The uncertainties are statistical 95% confidence limits. All of the quantities given in the upper part of this table were measured. The quantities given in the lower part of this table were calculated

| Expt. No. | direction of reaction | $T$ (K) | pH | $m$(ADP) ($mol\ kg^{-1}$) | $m(MgCl_2)$ | $m$(Tris) | $-\Delta H'_A$ ($kJ\ mol^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1 | forward | 298.15 | 8.37 | 0.005275 | 0.0002643 | 0.05021 | $1.20 \pm 0.41$ |
| 2 | forward | 304.55 | 8.20 | 0.004900 | 0.0002927 | 0.04977 | $0.79 \pm 0.51$ |
| 3 | forward | 310.25 | 8.10 | 0.005131 | 0.0002827 | 0.05021 | $1.43 \pm 0.51$ |

| Expt. No. | $I$ ($mol\ kg^{-1}$) | pMg | $\nu_H$ | $-\nu_H \Delta H_{ion}$ ($kJ\ mol^{-1}$) | $\Delta H^{ex}$ | $\Delta H_{corr}$ | $\Delta H_A°$ |
|---|---|---|---|---|---|---|---|
| 1 | 0.058 | 6.89 | −0.00493 | 0.23 | 0.52 | 0.28 | −2.23 |
| 2 | 0.056 | 6.90 | −0.00736 | 0.35 | 0.56 | 0.36 | −2.06 |
| 3 | 0.059 | 6.97 | −0.00886 | 0.42 | 0.61 | 0.35 | −2.81 |

value. The error estimate assigned to $\Delta H_A^\circ$ is largely attributable to the uncertainty in the correction for the blank heats of mixing which comprise a substantial correction to the measured reaction heats (see Section 2). The calculated values of $\nu_H$ ($-0.0049$ to $-0.0089$) given in Table 2 are consistent with the very small change in pH ($-0.001 \pm 0.018$) determined by a direct measurement with a pH meter. However, a value of $\nu_H$ equal to $0.01 \pm 0.006$ was calculated (see eq. 25 in ref. [1]) from the data in which $K_A'$ was determined as a function of pH. Thus, $\nu_H$ is uncertain by about $\pm 0.01$. This leads to a corresponding uncertainty of about $\pm 0.5$ kJ mol$^{-1}$ in $\Delta H_A^\circ$. This uncertainty is also included in the estimated error assigned to $\Delta H_A^\circ$ as well as an allowance for possible contributions due to errors in the proton and magnesium ion binding corrections and in the adjustment to the standard state. The enthalpy change determined from the calorimetric measurements is in agreement with the enthalpy change as obtained from the temperature dependency of the equilibrium data and an average value of $\Delta H_A^\circ = -(1.5 \pm 1.5)$ kJ mol$^{-1}$ at 298.15 K is adopted. Combination of the enthalpy and Gibbs energy changes leads to an entropy change of $-17 \pm 5$ J mol$^{-1}$ K$^{-1}$ at 298.15 K. In summary, the thermodynamic parameters at 298.15 K for the reference reaction are: $K_A^\circ = 0.225 \pm 0.010$, $\Delta G_A^\circ = 3.70 \pm 0.11$ kJ mol$^{-1}$, $\Delta H_A^\circ = -1.5 \pm 1.5$ kJ mol$^{-1}$, $\Delta S_A^\circ = -17 \pm 5$ J mol$^{-1}$ K$^{-1}$, and $\Delta C_{p,A}^\circ = -46 \pm 90$ J mol$^{-1}$ K$^{-1}$. A value of $B$ of 1.5 kg$^{1/2}$ mol$^{-1/2}$ has also been found to describe the variation of the equilibrium data with ionic strength.

Comparisons between measured and calculated values of the equilibrium constant $K_A'$ are shown in Figs. 2–5. All calculated values of $K_A'$, i.e. the solid curves in these figures, are based upon the assigned values of $K_A^\circ$, $\Delta H_A^\circ$, and $\Delta C_{p,A}^\circ$ determined in this study and the thermodynamic parameters for the hydrogen and magnesium-ion binding reactions given in Table 1 of ref. [1] and the model described in that paper. Examination of these figures shows that the measured and calculated values of $K_A'$ are in agreement within the indicated error limits. The dotted lines in these figures represent estimates of the uncertainties to be associated with the calculated values of $K_A'$.

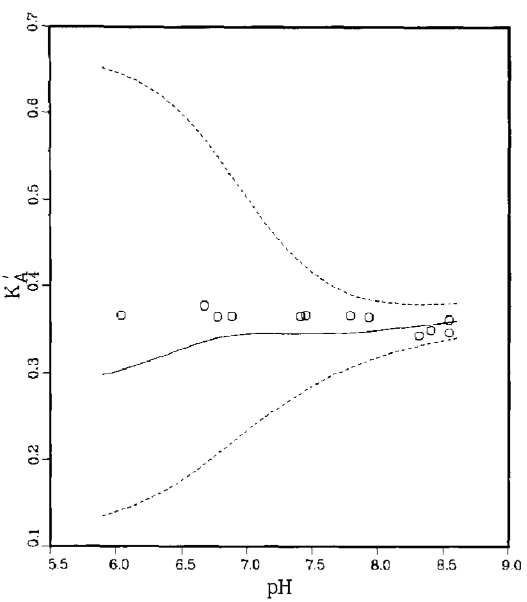


Fig. 2. Plot of the apparent equilibrium constant ($K_A'$) as a function of pH. The measured equilibrium constants (○) were obtained at 298.15 K, $6.3 \le \text{pMg} \le 7.2$, and $0.03 \le I \le 0.06$ mol kg$^{-1}$. The solid line is the calculated curve. The dashed lines are approximate limits of error in the calculated curve which were obtained by perturbing the assigned values of $K_A^\circ$ and the constants $pK_{1ATP}$, $pK_{1ADP}$, and $pK_{1AMP}$ by their assigned error estimates (see Table 1 in ref. [1]).

These uncertainties were obtained from the estimated errors associated with the parameters used in the model calculation.

In Fig. 4 the upturn in the calculated values of $K_A'$ at low pMg is attributable to the presence of $Mg_2ATP$. It was found that better agreement between measured and calculated values of $K_A'$ was obtained at low values of pMg if the presence of this species was neglected. However, this was not done since four studies indicate the existence of $Mg_2ATP$ and report equilibrium data that are in fair agreement (see Table 3 in ref. [1]). In spite of these substantial uncertainties, the results of the modelling calculations are gratifying in that the calculated results give either satisfactory or approximate representations of the dependency of the equilibrium data upon the environmental conditions which are normally varied in biochemical reactions, namely pH, pMg, temperature, and ionic strength. Clearly, care has to be exercised and large errors may result when equilibrium constants or other thermodynamic data are adjusted over wide ranges of environmental conditions. We are not aware of any other investigations where all of these comparisons have been made for a system possessing this degree of complexity.

Comparisons between the results of the earlier investigations and values of calculated values of $K'_A$ are given in Table 3. For the data of Lawson and Veech [12] the comparison is also made graphically in Fig. 6. This latter comparison is of particular interest since the results pertain to physiological conditions. Note that two of our data points (experiments 39 and 40 in Table 1 and shown as the triangles in Fig. 6) were determined under conditions very similar to those used by Lawson and Veech in their experiments. The total uncertainties in the apparent equilibrium constants reported by Lawson and Veech are estimated to be approximately five percent of the reported values [25], while our results are judged to be reliable within two percent. Thus, the results

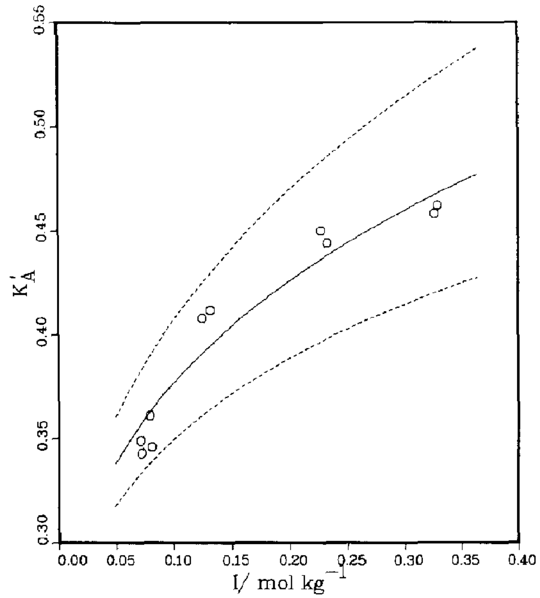


Fig. 3. Plot of the apparent equilibrium constant ($K'_A$) as a function of ionic strength. The measured equilibrium constants (○) were obtained at 298.15 K, $8.4 \leq pH \leq 8.9$, and $6.4 \leq pMg \leq 7.1$. The solid line is the calculated curve. The dashed lines are approximate limits of error in the calculated curve which were obtained by perturbing the assigned values of $K^{\circ}_A$ and of $B$ by their assigned error estimates.

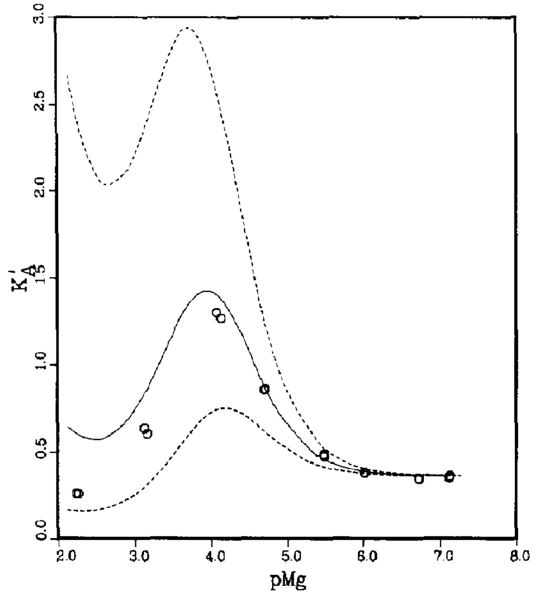


Fig. 4. Plot of the apparent equilibrium constant ($K'_A$) as a function of pMg. The measured equilibrium constants (○) were obtained at 298.15 K, $0.05 \leq I \leq 0.10$ mol kg$^{-1}$, and $8.1 \leq pH \leq 8.6$. The solid line is the calculated curve. The dashed lines are approximate limits of error in the calculated curve which were obtained by perturbing the assigned values of $K^{\circ}_A$ and the equilibrium constants for the magnesium ion binding reactions involving $ATP^{4-}$, $ADP^{3-}$, and $AMP^{2-}$ by their assigned error estimates (see Table 1 in ref. [1]).

of our experiments are in good agreement with those of Lawson and Veech.

Equilibrium data as a function of temperature have been reported by Bowen and Kerwin [4] and by Su and Russell [15]. We calculate values of $\Delta H^{\circ}_A$ equal to $3.2 \pm 3.4$ and $0.12 \pm 3.4$ kJ mol$^{-1}$ at 298.15 K from their [5,15] respective data. Here, we used a value of $B = 1.5$ kg$^{1/2}$ mol$^{-1/2}$, and the thermodynamic parameters for the hydrogen and magnesium-ion binding reactions given in Table 1 of ref. [1]. The uncertainties in the calculated enthalpy changes are equal to two standard deviations. These results are in agreement with the value which has been determined for $\Delta H^{\circ}_A$ in this study. The equilibrium data of Callaghan [6] and of Su and Russell [15] show a monotonic decrease of $K'_A$ with increasing pH which are in qualitative agreement with our measurements. The results of Blair [3] and of Bowen and Kerwin [5] show a peak in a plot of $K'_A$ as a function of pMg which

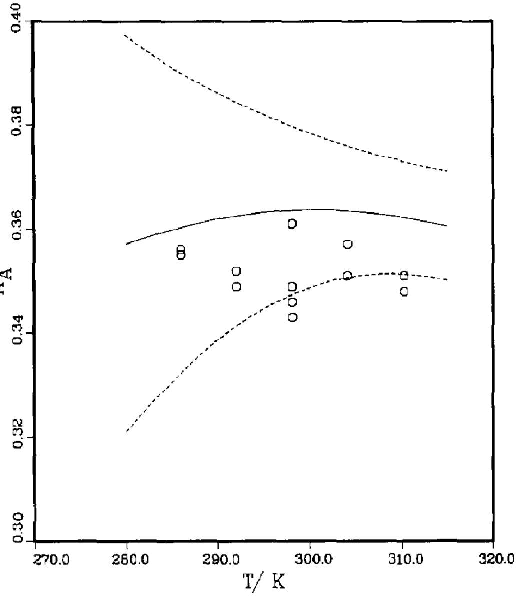


Fig. 5. Plot of the apparent equilibrium constant ($K'_A$) as a function of temperature. The measured equilibrium constants (○) were obtained at $8.0 \leq pH \leq 8.9$, $5.9 \leq pMg \leq 6.8$, and $0.07 \leq I \leq 0.08$ mol kg$^{-1}$. The solid line is the calculated curve. The dashed lines are approximate limits of error in the calculated curve which were obtained by perturbing the assigned values of $K^{\circ}_A$, $\Delta H^{\circ}_A$, and $\Delta C^{\circ}_{p,A}$ by their assigned error estimates.

is qualitatively similar to both the results of Lawson and Veech [12] and those obtained in this study. De Weer and Lowe [8] did not report their essential experimental data and give only a plot of $K'_A$ as a function of the calculated values of pMg. Thus, we were unable to recalculate their results as was done for the other studies summarized in Table 3.

The parameters in the equilibrium model are the thermodynamic quantities given in Table 1 in ref. [1] and the $B$ parameter used in the expression for the activity coefficients of the species in solution. In that table, only the values of $K^{\circ}$, $\Delta H^{\circ}$, $\Delta S^{\circ}$, and $\Delta C_p^{\circ}$ for the reference reaction were obtained from the equilibrium and calorimetric data given in this study. The other parameters in the model are p$K^{\circ}$, $\Delta H^{\circ}$, and $\Delta C_p^{\circ}$ for the binding of hydrogen and magnesium ions to the nucleotides. These values are based upon results from the literature and it is likely that some of these data are in error. Therefore, one must expect differences between the experimental data reported in this paper and the values calculated from the model for the overall reaction. Within the limits of error assigned to our own measurements, these differences are systematic (see Table 3 and Figs. 2, 4, 5, and 6), albeit within the limits of error of the calculated values. Thus, the model as applied in this paper should not be viewed as an exact method of calculating $K'_A$.

We believe that our measurements of $K'_A$ and $\Delta H'_A$ are correct within the overall error estimate of two percent. As noted above, our equilibrium data at 311.15 K and pMg 3.5 are also in agreement with the results of Lawson and Veech [12] which we also judge to be accurate. Since the total amount of magnesium was not reported by Ktalckar [11], Green et al. [10], and Bowen and Kerwin [4] it is not possible to comment upon the accuracy of their measurements. Also, since the results of several of the other studies were done under conditions significantly different than those used herein it is not possible to positively attribute the differences between measured and calculated values of $K'_A$ to either errors in the model or to the measurements themselves. Nevertheless, some of the differences between the calculated and measured values seem rather large for some of the data reported by Bowen and Kerwin [5], Callaghan [5], Callaghan and Weber [6], Atkinson et al. [2], Su and Russell [15], and Blair [3].

The results obtained in this study also have a relevance to the understanding of the thermodynamics of other phosphorylation reactions. For example, Alberty [26] relied largely upon the earlier measurements of Bowen and Kerwin [4] in his construction of Gibbs energy surfaces for several phosphorylation reactions. Thus, one can use the thermodynamic data for the hydrolysis of ATP:

$$ATP^{4-}(ao) + H_2O(liq) \rightleftharpoons ADP^{3-}(ao) + HPO_4^{2-}(ao) + H^+(ao) \quad (E)$$

These data can be combined with the results for the disproportionation reaction (A) to calculate thermodynamic parameters for the reaction:

$$ADP^{3-}(ao) + H_2O(liq) \rightleftharpoons AMP^{2-}(ao) + HPO_4^{2-}(ao) + H^+(ao) \quad (F)$$

Thus, combination of the results obtained in this study with the thermodynamic parameters given in Table II in the study by Gajewski et al. [27] leads to the following for reaction (F): $\Delta G^\circ = 7.1$ kJ mol$^{-1}$, $\Delta H^\circ = -22.0$ kJ mol$^{-1}$, $\Delta S^\circ = -98$ J mol$^{-1}$ K$^{-1}$, and $\Delta C_p^\circ \approx -283$ J mol$^{-1}$ K$^{-1}$ at 298.15 K. There are no direct calorimetric or equilibrium measurements for this reaction. However, Hinz et al. [28] have performed calorimetric measurements which led to enthalpy changes associated with the hydrolysis of the $\alpha$, $\beta$, and $\gamma$ bonds of the guanosine phosphates:

$$GMP^{2-}(ao) + H_2O(liq) \rightleftharpoons \text{guanosine}(ao) + HPO_4^{2-}(ao) \quad (G)$$

$$GDP^{3-}(ao) + H_2O(liq) \rightleftharpoons GMP^{2-}(ao) + HPO_4^{2-}(ao) + H^+(ao) \quad (H)$$

$$GTP^{4-}(ao) + H_2O(liq) \rightleftharpoons GDP^{3-}(ao) + HPO_4^{2-}(ao) + H^+(ao) \quad (I)$$

Their [28] results for the enthalpy changes for reactions (G), (H), and (I) are, respectively, 2.7, −23.0, and −23.1 kJ mol$^{-1}$. Combination of the enthalpy changes for reactions (H) and (I) lead to $\Delta H^\circ = 0.0$ kJ mol$^{-1}$ for:

$$2\,GDP^{3-}(ao) \rightleftharpoons GMP^{2-}(ao) + GTP^{4-}(ao) \quad (J)$$

Thus, the findings of Hinz et al. [28] that the enthalpy changes for the hydrolysis of the $\beta$ and $\gamma$ bonds are both moderately negative and approximately equal and that the enthalpy change corresponding to the hydrolysis of the $\alpha$ bond is small are in agreement with the results of this study.

In a pioneering paper, Rose [29] used the observed dependency of the apparent equilibrium constant $K'_A$ on pMg to calculate the free magnesium ion concentration in human red blood cells. To do this, he determined the equilibrium constant $K'_A = 0.77$ in such cells at 308.15 K. Veech et al. [30] later reported a value of $0.784 \pm 0.037$ at 311.15 K. These two results are in excellent agreement. In his calculation leading to a value of the free magnesium ion concentration in human red blood cells, Rose [29] relied upon the earlier results of Bowen and Kerwin [5] for $K'_A$ and a limited set of the magnesium ion – nucleotide binding constants available at the time he did his research. Use of the calculated curve, i.e. the solid line, in Fig. 6 together with a value of $K'_A = 0.78$ in human red blood cells leads to a value of pMg of 4.31. The same value is obtained from an empirical curve drawn through the data of Lawson and Veech [12]. In his paper, Rose [29] gave several arguments against the selection of a lower value of pMg such as could be obtained from the functional dependency of $K'_A$ on pMg. The selection of a lower value of pMg can be completely ruled out, however, by a knowledge of the total concentrations of the nucleotides [29] and of the total amount of magnesium in human red blood cells [31] coupled with the magnesium ion – nucleotide binding constants [1]. With the empirical curve of Lawson and Veech [12] and a calculated ($T = 311.15$ K, $I = 0.25$ mol kg$^{-1}$, and $B = 1.5$ kg$^{1/2}$ mol$^{-1/2}$) activity coefficient of 0.253 for $Mg^{2+}$, a value of $1.94 \times 10^{-4}$ mol kg$^{-1}$ is obtained for the molality of $Mg^{2+}$ in human red blood cells. The value obtained by Rose [29] from the earlier thermodynamic results was approximately $1.34 \times 10^{-4}$ mol kg$^{-1}$. The molality of free magnesium ion in human red blood cells is substantially lower than the values reported for it in rat liver, brain, and kidney. In these latter systems, values ranging from $4.6 \times 10^{-4}$ to $1.34 \times 10^{-3}$ mol kg$^{-1}$ have been reported [32,33]. It should be noted that in all of these calculations and studies, the values obtained for the molalities of the free magnesium ion are dependent upon the knowledge of thermodynamic equilibrium constants. These equilibrium constants in turn are generally functions of pH and ionic strength. These dependencies and the difficulties and uncertainties associated with the knowledge of the exact ionic strength and pH appropriate to solutions taken from living systems create additional problems in the calculation of the free magnesium ion concentrations. The proper establishment of the pH and ionic strength appropriate for living systems are also similarly dependent upon a knowledge of equilibrium constants and of the other environmental parameters. Thus, a general solution to the establishment of physiological conditions would require a self-consistent approach with respect to the

Table 3

Equilibrium measurements for the disproportionation of ADP to AMP to ATP as reported in the literature and compared with values calculated from the equilibrium model, the values of $K_A^\circ$, $\Delta H_A^\circ$, and $\Delta C_{p,A}^\circ$ determined in this study, a value of $B = 1.5$ kg$^{1/2}$ mol$^{-1/2}$, and the magnesium and hydrogen ion binding data in Table 1 of ref. [1]. The total magnesium was not reported in the studies of Kalckar [11], Green et al. [10], and Bowen and Kerwin [4]. In performing calculations on the results of these three studies [4,10,11], we have assumed that pMg was ≥10

| $T$ (K) | pH | pMg | $I$ (mol kg$^{-1}$) | $K'_A$(meas.) | $K'_A$(calc.) | Reference |
|---|---|---|---|---|---|---|
| 303.15 | 7.50 | – | 0.0072 | 0.300 | 0.296 | Kalckar [11] |
| 298.15 | 7.40 | 2.75 | 0.0316 | 0.444 | 0.565 | Eggleston and Hems [9] |
| 300.15 | 6.70 | – | 0.0055 | 0.451 | 0.289 | Green et al. [10] |
| 298.15 | 7.00 | 4.56 | 0.0368 | 1.000 | 0.850 | Siekevitz and Potter [14] |
| 274.15 | 6.00 | – | 0.0004 | 0.383 | 0.238 | Bowen and Kerwin [4] |
| 293.15 | 6.00 | – | 0.0005 | 0.394 | 0.236 | |
| 313.15 | 6.00 | – | 0.0011 | 0.478 | 0.231 | |
| 293.15 | 6.00 | 7.00 | 0.0041 | 0.368 | 0.256 | Bowen and Kerwin [5] |
| 293.15 | 6.00 | 6.00 | 0.0041 | 0.358 | 0.261 | |
| 293.15 | 6.00 | 4.94 | 0.0040 | 0.523 | 0.315 | |
| 293.15 | 6.00 | 4.75 | 0.0040 | 0.958 | 0.346 | |
| 293.15 | 6.00 | 4.33 | 0.0039 | 1.239 | 0.467 | |
| 293.15 | 6.00 | 3.93 | 0.0040 | 1.094 | 0.668 | |
| 293.15 | 6.00 | 3.48 | 0.0046 | 0.761 | 0.906 | |
| 293.15 | 6.00 | 3.18 | 0.0058 | 0.677 | 0.960 | |
| 293.15 | 6.00 | 2.93 | 0.0081 | 0.474 | 0.937 | |
| 303.15 | 6.12 | 3.18 | 0.0440 | 0.690 | 0.997 | Callaghan [6] |
| 303.15 | 6.81 | 3.41 | 0.0481 | 0.470 | 1.055 | |
| 303.15 | 7.50 | 3.49 | 0.0625 | 0.460 | 1.137 | |
| 303.15 | 7.88 | 3.49 | 0.0771 | 0.400 | 1.161 | |
| 313.15 | 7.50 | 4.56 | 0.0188 | 0.426 | 1.370 | Callaghan and Weber [7] |
| 313.15 | 7.50 | 3.14 | 0.0120 | 0.364 | 0.543 | |
| 298.15 | 7.00 | 2.88 | 0.2854 | 0.476 | 0.957 | Atkinson et al. [2] |
| 298.15 | 7.00 | 2.35 | 0.3240 | 0.278 | 0.753 | |
| 303.15 | 7.40 | 3.28 | 0.0257 | 0.811 | 0.814 | Markland and Wadkins [13] |
| 303.15 | 5.00 | 2.08 | 0.0971 | 0.496 | 1.146 | Su and Russell [15] |
| 303.15 | 6.00 | 2.12 | 0.0904 | 0.418 | 0.911 | |
| 303.15 | 7.00 | 2.16 | 0.0993 | 0.269 | 0.657 | |
| 303.15 | 8.00 | 2.22 | 0.1628 | 0.236 | 0.632 | |
| 303.15 | 9.00 | 2.27 | 0.2343 | 0.205 | 0.653 | |
| 303.15 | 10.00 | 2.28 | 0.2501 | 0.197 | 0.659 | |
| 303.15 | 7.00 | 3.51 | 0.0513 | 0.640 | 1.127 | |
| 303.15 | 7.00 | 2.66 | 0.0595 | 0.413 | 0.611 | |
| 303.15 | 7.00 | 2.16 | 0.0993 | 0.245 | 0.657 | |
| 303.15 | 7.00 | 1.84 | 0.1914 | 0.216 | 0.855 | |
| 322.60 | 7.00 | 2.21 | 0.1183 | 0.245 | 0.624 | |
| 300.40 | 7.00 | 2.16 | 0.0968 | 0.272 | 0.660 | |
| 280.30 | 7.00 | 2.12 | 0.0897 | 0.303 | 0.708 | |
| 298.15 | 7.50 | 6.74 | 0.0043 | 0.333 | 0.310 | Blair [3] |
| 298.15 | 7.50 | 4.86 | 0.0038 | 0.567 | 1.179 | |
| 298.15 | 7.50 | 3.97 | 0.0039 | 0.817 | 1.368 | |
| 298.15 | 7.50 | 3.97 | 0.0039 | 0.877 | 1.368 | |
| 298.15 | 7.50 | 3.53 | 0.0045 | 1.167 | 0.896 | |
| 298.15 | 7.50 | 3.53 | 0.0045 | 1.193 | 0.896 | |
| 298.15 | 7.50 | 3.53 | 0.0045 | 1.317 | 0.896 | |
| 298.15 | 7.50 | 3.24 | 0.0056 | 1.307 | 0.659 | |
| 298.15 | 7.50 | 3.24 | 0.0056 | 1.320 | 0.659 | |
| 298.15 | 7.50 | 3.24 | 0.0056 | 1.400 | 0.659 | |

Table 3 (continued)

| $T$ (K) | pH | pMg | $I$ (mol kg$^{-1}$) | $K'_A$(meas.) | $K'_A$(calc.) | Reference |
|---|---|---|---|---|---|---|
| 298.15 | 7.50 | 2.06 | 0.0647 | 0.900 | 0.667 | Blair [3] (continued) |
| 298.15 | 7.50 | 2.66 | 0.0136 | 0.543 | 0.486 | |
| 298.15 | 7.50 | 6.11 | 0.0992 | 0.433 | 0.394 | |
| 298.15 | 7.50 | 4.55 | 0.0989 | 0.637 | 0.819 | |
| 298.15 | 7.50 | 4.13 | 0.0991 | 0.833 | 1.148 | |
| 298.15 | 7.50 | 3.84 | 0.0995 | 1.153 | 1.288 | |
| 298.15 | 7.50 | 3.54 | 0.1006 | 1.367 | 1.237 | |
| 298.15 | 7.50 | 3.34 | 0.1020 | 1.167 | 1.110 | |
| 298.15 | 7.50 | 3.18 | 0.1036 | 1.000 | 0.991 | |
| 298.15 | 7.50 | 2.91 | 0.1085 | 0.600 | 0.794 | |
| 2108 | | | | | | |
| 311.15 | 6.91 | 3.49 | 0.250 | 0.980 | 1.129 | Lawson and Veech [12] |
| 311.15 | 6.90 | 3.49 | 0.250 | 0.982 | 1.128 | |
| 311.15 | 6.91 | 3.48 | 0.250 | 0.983 | 1.129 | |
| 311.15 | 6.89 | 3.48 | 0.250 | 0.964 | 1.127 | |
| 311.15 | 6.98 | 3.98 | 0.250 | 0.946 | 0.975 | |
| 311.15 | 6.98 | 3.98 | 0.250 | 0.871 | 0.975 | |
| 311.15 | 6.97 | 3.96 | 0.250 | 0.981 | 0.984 | |
| 311.15 | 6.97 | 3.96 | 0.250 | 0.929 | 0.984 | |
| 311.15 | 6.99 | 4.54 | 0.250 | 0.708 | 0.660 | |
| 311.15 | 6.99 | 4.14 | 0.250 | 0.899 | 0.879 | |
| 311.15 | 6.99 | 3.89 | 0.250 | 1.014 | 1.027 | |
| 311.15 | 6.99 | 3.72 | 0.250 | 1.044 | 1.106 | |
| 311.15 | 6.99 | 3.59 | 0.250 | 1.060 | 1.136 | |
| 311.15 | 6.99 | 3.21 | 0.250 | 0.842 | 1.054 | |
| 311.15 | 6.99 | 2.86 | 0.250 | 0.572 | 0.851 | |
| 311.15 | 6.99 | 2.53 | 0.250 | 0.380 | 0.704 | |
| 298.15 | 8.31 | 6.72 | 0.072 | 0.343 | 0.361 | Tewari et al. (this study) |
| 298.15 | 8.54 | 6.71 | 0.081 | 0.346 | 0.366 | |
| 286.05 | 8.68 | 6.59 | 0.072 | 0.355 | 0.364 | |
| 286.05 | 8.85 | 6.59 | 0.080 | 0.356 | 0.369 | |
| 292.15 | 8.46 | 6.66 | 0.071 | 0.352 | 0.362 | |
| 292.15 | 8.67 | 6.65 | 0.080 | 0.349 | 0.368 | |
| 304.15 | 8.16 | 6.78 | 0.072 | 0.357 | 0.359 | |
| 304.15 | 8.34 | 6.78 | 0.080 | 0.351 | 0.363 | |
| 310.25 | 7.98 | 6.84 | 0.071 | 0.351 | 0.356 | |
| 310.25 | 8.13 | 6.84 | 0.078 | 0.348 | 0.360 | |
| 298.15 | 8.34 | 6.02 | 0.070 | 0.381 | 0.385 | |
| 298.15 | 8.54 | 6.02 | 0.080 | 0.378 | 0.388 | |
| 298.15 | 8.28 | 4.70 | 0.061 | 0.861 | 0.883 | |
| 298.15 | 8.51 | 4.69 | 0.068 | 0.854 | 0.870 | |
| 298.15 | 8.40 | 2.24 | 0.093 | 0.260 | 0.601 | |
| 298.15 | 8.56 | 2.27 | 0.096 | 0.258 | 0.597 | |
| 298.15 | 8.33 | 3.12 | 0.056 | 0.634 | 0.816 | |
| 298.15 | 8.53 | 3.16 | 0.062 | 0.603 | 0.866 | |
| 298.15 | 8.30 | 5.49 | 0.069 | 0.475 | 0.457 | |
| 298.15 | 8.50 | 5.48 | 0.076 | 0.484 | 0.458 | |
| 298.15 | 8.32 | 4.07 | 0.057 | 1.302 | 1.418 | |
| 298.15 | 8.50 | 4.13 | 0.063 | 1.268 | 1.368 | |
| 298.15 | 8.40 | 7.12 | 0.071 | 0.349 | 0.356 | |
| 298.15 | 8.54 | 7.12 | 0.079 | 0.361 | 0.362 | |
| 298.15 | 8.49 | 6.92 | 0.125 | 0.408 | 0.391 | |
| 298.15 | 8.67 | 6.93 | 0.132 | 0.412 | 0.394 | |
| 298.15 | 8.57 | 6.67 | 0.228 | 0.450 | 0.435 | |

Table 3 (continued)

| $T$ (K) | pH | pMg | $I$ (mol kg$^{-1}$) | $K'_A$(meas.) | $K'_A$(calc.) | Reference |
|---|---|---|---|---|---|---|
| 298.15 | 8.73 | 6.70 | 0.233 | 0.444 | 0.437 | Tewari et al. (this study, continued) |
| 298.15 | 8.68 | 6.46 | 0.328 | 0.462 | 0.467 | |
| 298.15 | 8.87 | 6.46 | 0.326 | 0.458 | 0.466 | |
| 298.15 | 7.79 | 7.13 | 0.052 | 0.366 | 0.346 | |
| 298.15 | 7.93 | 7.15 | 0.061 | 0.364 | 0.351 | |
| 298.15 | 6.04 | 6.34 | 0.026 | 0.366 | 0.302 | |
| 298.15 | 6.67 | 6.84 | 0.037 | 0.377 | 0.339 | |
| 298.15 | 6.77 | 6.97 | 0.034 | 0.364 | 0.339 | |
| 298.15 | 6.88 | 7.05 | 0.038 | 0.365 | 0.344 | |
| 298.15 | 7.41 | 7.22 | 0.044 | 0.365 | 0.345 | |
| 298.15 | 7.45 | 7.24 | 0.048 | 0.366 | 0.347 | |
| 311.15 | 6.83 | 3.51 | 0.249 | 0.934 | 1.117 | |
| 311.15 | 7.04 | 3.54 | 0.245 | 0.928 | 1.148 | |

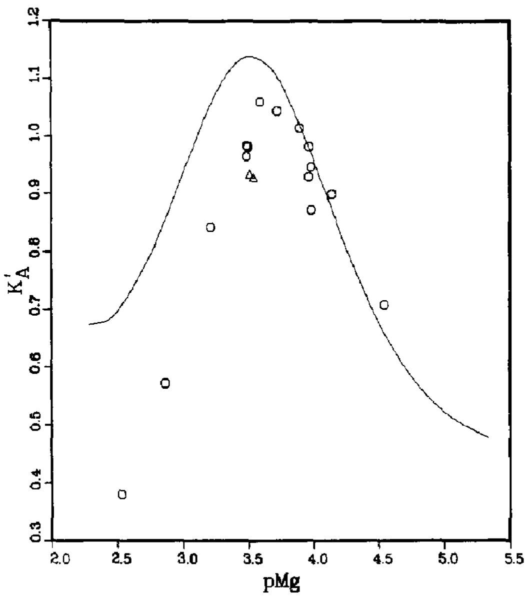


Fig. 6. Plot of the apparent equilibrium constants ($K'_A$) as a function of pMg at 311.15 K and at $6.83 \leq \text{pH} \leq 7.04$ and $I = 0.25$ mol kg$^{-1}$. The data of Lawson and Veech [12] are given by (○), the two data points determined in this investigation by (△). The solid line was calculated from the equilibrium model, the values of $K^\circ_A$, $\Delta H^\circ_A$, $\Delta C^\circ_{p,A}$, and $B$ determined in this study, and the magnesium- and hydrogen-ion binding data are those given in Table 1 of ref. [1].

treatment of pH, ionic strength, and the free magnesium-ion concentration.


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## Note added in proof

Following completion of this research two additional publications dealing with the thermodynamics of the disproportionation reaction of ADP to AMP and ATP were brought to our attention. They are:

R.S. Langer, C.R. Gardner, B.K. Hamilton and C.K. Colton, AIChE J. 23 (1977) 1; the equilibrium data shown graphically in this paper are given in tabular form in the following thesis: R.S. Langer, Enzymatic Regulation of ATP, Massachusetts Institute of Technology, Cambridge, MA (1974).

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