Biophysical Chemistry 4 (1976) 215–221

# THERMODYNAMICS OF HEXOKINASE CATALYZED REACTIONS.

## II. Measurement and calculation of enthalpies of reaction as a function of magnesium ion concentration

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Received 2 December 1975

Enthalpies of phosphorylation of glucose by adenosine 5'-triphosphate have been measured as a function of concentrations of magnesium chloride in TRIS/TRIS • HCl buffer in the pH range 8.64 to 8.98. These measurements are compared with the results of calculations of these enthalpies that use a coupled equilibrium formalism with equilibrium data and enthalpy values selected from the literature. The experimental results span the range of magnesium ion concentrations $1 \times 10^{-6}$ to 0.3 mol $\ell^{-1}$ and show a total variation in the enthalpy of reaction of almost 10 kJ mol$^{-1}$, with the most exotherm reaction occurring at a magnesium ion concentration of $6.0 \times 10^{-4}$ mol $\ell^{-1}$. The calculated enthalpies of reaction, except for the magnesium ion concentration range $4 \times 10^{-6}$ to $5 \times 10^{-4}$ mol $\ell^{-1}$, are, within estimated uncertainty intervals (0.8 to 10.2 kJ mol$^{-1}$), in agreement with the measured values.

## 1. Introduction[*]

A detailed understanding of the thermodynamics of reactions in which ATP is a participant requires an understanding of the effects on measurable quantities due to variations in pH and pMg[**]. While in principle these effe may be calculated [1–4], we are aware of few instances where, for biochemical systems, comparisons have been made between the predictions of a coupled equilibrium formalism and experimental data.

In this report, we have extended our previous measurements [5] of enthalpies of reaction for the *hexokinase* catalyzed phosphorylation of glucose by ATP by examining the effects of varying concentrations of magnesium chloride. These measurements are then compared with the results of calculations that use a coupled equilibrium formalism and the appropriate pK and $\Delta H$ values from the literature as input data.

A comparison of this sort is also of importance in that biochemical reactions may occur under a wider variety of conditions than can practically be measured. However, if there exist reliable thermodynamic data for the specific ionic reactions that contribute to the overall process, it then becomes possible to calculate the desired quantities.

## 2. Experimental

The experimental procedures and materials used were the same as those used and more completely described previously [5]. The di-TRIS salt of ATP (from Sigma Chemical Company[‡], assay 97%) was used in all experimer

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[*] Abbreviations used in this paper are: ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; TRIS, tris(hydroxyme aminomethane; G6P, glucose 6-phosphate.

[**] pMg is defined by the relationship: pMg = $-\log_{10}(\text{Mg}^{2+})/\text{mol}\ \ell^{-1}$.

[‡] Certain commercial materials and products are identified in this paper in order to specify the experimental procedure adequat Such identification does not imply recommendation or endorsement by the National Bureau of Standards.

Table 1
Compositions of solutions with results of experiments and calculations on the ATP-glucose/*hexokinase* reaction as a function of pMg. Temperature is 25.01±0.02°C

| Series | Glucose[b] (mmol/kg solution) | ATP (mmol/kg solution) | TRIS (mol/kg solution) | $MgCl_2$ (mmol/kg solution) | Enzyme (g/kg solution) | pH | $pMg_{CALC}$[a] | ionic strength[a] (mol/ℓ) | $-\Delta H^\circ_{OBS}$ (measured)[c] (kJ/mol) | $-\Delta H^\circ_{OBS}$ (calculated)[a] (kJ/mol) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10.269 | 17.474 | 0.47843 | 322.45 | 2.841 | 8.85 | 0.51 | 1.01 | 67.78±0.10 | 67.7±10.2 |
| 2 | 8.7595 | 15.095 | 0.47802 | 257.91 | 1.183 | 8.90 | 0.62 | 0.78 | 67.02±0.52 | 67.7±10.1 |
| 3 | 10.858 | 18.230 | 0.49663 | 156.69 | 0.9668 | 8.86 | 0.90 | 0.45 | 69.98±0.29 | 67.8±10.1 |
| 4 | 10.147 | 18.102 | 0.50276 | 100.03 | 0.9514 | 8.90 | 1.17 | 0.27 | 71.29±0.64 | 68.1±10.0 |
| 5 | 7.4136 | 22.443 | 0.48381 | 54.460 | 0.6551 | 8.92 | 1.86±0.02 | 0.11 | 73.88±0.16 | 69.9± 9.1 |
| 6 | 14.011 | 19.905 | 0.49788 | 34.615 | 1.113 | 8.86 | 2.36±0.07 | 0.09 | 74.72±0.05 | 72.8± 7.7 |
| 7 | 12.429 | 19.476 | 0.49835 | 29.227 | 0.8475 | 8.87 | 2.59±0.08 | 0.09 | 75.21±0.21 | 74.5± 6.8 |
| 8 | 13.992 | 19.760 | 0.51018 | 21.187 | 1.121 | 8.88 | 3.02±0.08 | 0.10 | 76.57±0.13 | 78.1± 5.0 |
| 9 | 7.7066 | 19.511 | 0.50079 | 18.708 | 0.9617 | 8.90 | 3.10±0.05 | 0.10 | 76.72±0.17 | 78.8± 4.7 |
| 10 | 13.266 | 19.733 | 0.51059 | 18.040 | 1.199 | 8.89 | 3.22±0.07 | 0.10 | 76.74±0.13 | 79.8± 4.2 |
| 11 | 10.799 | 18.403 | 0.51153 | 15.560 | 1.0918 | 8.64 | 3.37±0.07 | 0.15 | 76.39±0.14 | 80.4± 3.7 |
| 12 | 11.799 | 18.474 | 0.51121 | 14.020 | 0.7699 | 8.92 | 3.55±0.07 | 0.11 | 75.62±0.22 | 81.9± 3.0 |
| 13 | 8.4565 | 18.707 | 0.50299 | 10.589 | 0.8269 | 8.92 | 3.97±0.06 | 0.11 | 73.93±0.26 | 82.7± 1.9 |
| 14 | 10.677 | 19.311 | 0.51234 | 4.5303 | 0.8654 | 8.93 | 4.64±0.08 | 0.14 | 72.62±0.25 | 78.5± 1.4 |
| 15 | 8.4831 | 19.311 | 0.48827 | 1.2736 | 1.040 | 8.98 | 5.45±0.09 | 0.15 | 72.29±0.14 | 73.2± 0.9 |
| 16 | 14.178 | 21.332 | 0.51167 | 0.270[d] | 1.879 | 8.98 | 6.01±0.08 | 0.16 | 71.73±0.54 | 71.9± 0.8 |

[a] Calculated quantities, See section on Results and Discussion for method of computation and basis of estimated uncertainties. If for the quantity $pMg_{CALC}$, no uncertainty is given, the total uncertainty was estimated to be less than 0.01 pMg unit. For all calculated quantities, the numerical results refer to the conditions existing in the solutions following reaction.

[b] The solution compositions in this table (columns 2 to 6) have, except for column 6, units of mol/(kg solution) and may be related to the molarity scale of concentration by multiplication by the densities of the respective solutions, which were found to vary from 1.026 g $ml^{-1}$ for zero magnesium chloride to 1.050 g $ml^{-1}$ at a magnesium chloride concentration of 0.33 mol/(kg solution). Molarities were used in the computation of $pMg_{CALC}$, ionic strength, and $\Delta H_{OBS}$ (calculated).

[c] Stated uncertainty is the imprecision calculated on the basis of two estimated standard deviations of the mean. Total systematic error in the heat measurements is estimated to be no greater than 0.3 percent of the total heat measured. Three measurements were performed for each series, except for series 2, 9, and 12 where two measurements were performed.

[d] Estimated on basis of results of atomic-absorption analyses [5].

in order to avoid complications due to the presence of metal ions other than magnesium. Due to its possible instability, samples were always used within a month of receipt. Fisher certified magnesium chloride hexahydrate (assay 98.7%, major impurity excess moisture) and TRIS were used throughout, as was lyophilized yeast *hexokinase* from Calbiochem. All measurements were performed in aqueous solution at a temperature of 25.01±0.02°C.

The experimental data are presented in detail in table 1. The data in columns 2 thru 5 give the compositions of the substrate solutions prior to reaction; as before [5], for each substrate solution, an enzyme solution was prepared by pre-reaction of a portion of the substrate solution by introducing a measured quantity of *hexokinase* (see column 6). The quantities of these two solutions reacted in the calorimeter, although not given in table 1, were similar to those used in the previous report [5]. The columns labeled "pMg" and "*I*" (ionic strength) refer, respectively, to values of these parameters calculated using pK values selected from the literature and a combined-iterative computational procedure (see section 3). Attempts to perform measurements at values of pMg less than 0.5 were not successful due to serious loss of enzymatic activity. This observation is consistent with both the existence of the $Mg_2ATP^\circ$ species [6] and the hypothesis that, for this system, the active species of ATP is the ion $MgATP^{2-}$ (as was found to be the case for the creatine kinase reaction [7]). It should be noted that all measurements were carried out at sufficiently high pH that minor variations in pH should not significantly affect the quantity $\Delta H_{OBS}$. As previously, we have presented the experimental results in detail so that the data may still be reevaluated if additional information bearing on its interpretation later comes available.

Table 2
Selected values of thermodynamics constants used in calculations. Temperature is 25.0°C

| Process | Constant | pK[a)] | $\Delta H^\circ$ (kJ mol$^{-1}$) | References |
|---|---|---|---|---|
| $HATP^{3-} = H^+ + ATP^{4-}$ | $K_{1ATP}$ | $(7.68 - 3.56 I^{1/2} + 4.90 I) \pm 0.04$ | $-\ 7.0 \pm 1.3^{A}$ | [12] |
| $H_2ATP^{2-} = H^+ + HATP^{3-}$ | $K_{2ATP}$ | $4.00 \pm 0.10^{b)}$ in 0.15 M NaCl | $0 \pm 4^{b)A}$ | [2] |
| $HADP^{2-} = H^+ + ADP^{3-}$ | $K_{1ADP}$ | $(7.20 - 2.54 I^{1/2} + 3.84 I) \pm 0.04$ | $-\ 5.7 \pm 1.3^{A}$ | [12] |
| $H_2ADP^{1-} = H^+ + HADP^{2-}$ | $K_{2ADP}$ | $3.95 \pm 0.10^{b)}$ in 0.15 M NaCl | $+\ 1.0 \pm 4^{b)A}$ | [2] |
| $HG6P^{1-} = H^+ + G6P^{2-}$ | $K_{G6P}$ | $6.55 \pm 0.2$ at $I = 0$ | $-\ 1.8 \pm 4.0^{b)A}$ | Estimated [8 |
| $MgATP^{2-} = Mg^{2+} + ATP^{4-}$ | $K_{MgATP}$ | $\left(5.83 - 6.10 I^{1/2} + 8.74 I - \dfrac{2.04 I^{1/2}}{1 + 6.02 I^{1/2}}\right) \pm 0.10$ | $-18.7 \pm 1.0^{b)B}$ | [12,13] |
| $MgHATP^{1-} = Mg^{2+} + HATP^{3-}$ | $K_{MgHATP}$ | $\left(3.59 - 4.06 I^{1/2} + 6.36 I - \dfrac{2.04 I^{1/2}}{1 + 6.02 I^{1/2}}\right) \pm 0.12$ | $-\ 9.2 \pm 5.2^{A}$ | [12] |
| $Mg_2ATP^\circ = Mg^{2+} + MgATP^{2-}$ | $K_{Mg_2ATP}$ | $2.61 \pm 0.10^{b)}$ ($I = 0.005$ to $0.10$) | $-3 \pm 8$ (estimated, see results and Discussion) | [6] |
| $MgADP^{1-} = Mg^{2+} + ADP^{3-}$ | $K_{MgADP}$ | $\left(4.27 - 4.06 I^{1/2} + 6.36 I - \dfrac{2.04 I^{1/2}}{1 + 6.02 I^{1/2}}\right) \pm 0.10$ | $-13.2 \pm 1.0^{b)A}$ | [12,13] |
| $MgHADP^\circ = Mg^{2+} + HADP^{2-}$ | $K_{MgHADP}$ | $\left(2.45 - 2.03 I^{1/2} + 3.34 I - \dfrac{2.04 I^{1/2}}{1 + 6.02 I^{1/2}}\right) \pm 0.20$ | $-\ 3.8 \pm 5.9^{A}$ | [12] |
| $MgG6P = Mg^{2+} + G6P^{2-}$ | $K_{MgG_6P}$ | $2.48 \pm 0.2$ at $I = 0$ | $-12.2 \pm 2.0^{b)A}$ | Estimated [9 |
| $TRIS \cdot H^+ = H^+ + TRIS^\circ$ | $K_{TRIS}$ | $8.072 \pm 0.004$ at $I = 0$ | $-47.48 \pm 0.03^{B}$ | [14,15,16] |
| $Glucose + ATP^{4-} = G6P^{2-} + ADP^{3-} + H^+$ | $K_B$ | $\Delta G^\circ = +16.7$ kJ mol$^{-1}$ | $-23.8 \pm 0.7^{B}$ | [5] |

a) $pK = -\log_{10} K$, where $K$ has units of $\text{mol}\,\ell^{-1}$.
b) Indicates estimate of uncertainty on the part of this author.
A Van't Hoff enthalpy. B Calorimetric enthalpy.

## 3. Results and discussion

### 3.1. Coupled equilibrium analysis

For the process

$$\text{eq-D-glucose} + \Sigma\text{ATP} = \Sigma\text{eq-D-G6P} + \Sigma\text{ADP} + n_H H^+ ,$$

occurring in the presence of magnesium ions, it is possible to show that $\Delta H_A^\circ$ is given by the relationship (see 2 and below for explanation of notation):

$$\begin{aligned}
\Delta H_A^\circ = \Delta H_B^\circ + f_{ATP} \Bigg\{ & \frac{(Mg^{2+})}{K_{MgATP}} \Delta H_{MgATP}^\circ + \frac{(H^+)}{K_{1ATP}} \Delta H_{1ATP}^\circ + \frac{(H^+)(Mg^{2+})}{K_{1ATP} K_{MgHATP}} (\Delta H_{1ATP}^\circ + \Delta H_{MgHATP}^\circ) \\
& + \frac{(H^+)^2}{K_{1ATP} K_{2ATP}} (\Delta H_{1ATP}^\circ + \Delta H_{2ATP}^\circ) + \frac{(Mg^{2+})^2}{K_{MgATP} K_{Mg_2ATP}} (\Delta H_{MgATP}^\circ + \Delta H_{Mg_2ATP}^\circ) \Bigg\} \\
& - f_{ADP} \Bigg\{ \frac{(Mg^{2+})}{K_{MgADP}} \Delta H_{MgADP}^\circ + \frac{(H^+)}{K_{1ADP}} \Delta H_{1ADP}^\circ + \frac{(H^+)(Mg^{2+})}{K_{1ADP} K_{MgHADP}} (\Delta H_{1ADP}^\circ + \Delta H_{MgHADP}^\circ) \\
& + \frac{(H^+)^2}{K_{1ADP} K_{2ADP}} (\Delta H_{1ADP}^\circ + \Delta H_{2ADP}^\circ) \Bigg\} - f_{G6P} \Bigg\{ \frac{(Mg^{2+})}{K_{MgG6P}} \Delta H_{MgG6P}^\circ + \frac{(H^+)}{K_{G6P}} \Delta H_{G6P}^\circ \Bigg\} .
\end{aligned}$$

$\Delta H^\circ_B$ is the molar enthalpy change for the reference reaction

$$\text{eq-D-glucose} + \text{ATP}^{4-} = \text{eq-D-G6P}^{2-} + \text{ADP}^{3-} + \text{H}^+ \ . \tag{B}$$

The fractions of ATP, ADP, and G6P existing in the most basic forms (i.e., $\text{ATP}^{4-}$, $\text{ADP}^{3-}$, and $\text{G6P}^{2-}$) are given by

$$f_{\text{ATP}} = \left\{\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgATP}}} + \frac{(\text{Mg}^{2+})^2}{K_{\text{MgATP}}K_{\text{Mg}_2\text{ATP}}}\right) + \frac{(\text{H}^+)}{K_{1\text{ATP}}}\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgHATP}}}\right) + \frac{(\text{H}^+)^2}{K_{1\text{ATP}}K_{2\text{ATP}}}\right\}^{-1} , \tag{2}$$

$$f_{\text{ADP}} = \left\{\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgADP}}}\right) + \frac{(\text{H}^+)}{K_{1\text{ADP}}}\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgHADP}}}\right) + \frac{(\text{H}^+)^2}{K_{1\text{ADP}}K_{2\text{ADP}}}\right\}^{-1} , \tag{3}$$

$$f_{\text{G6P}} = \left\{1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgG6P}}} + \frac{(\text{H}^+)}{K_{\text{G6P}}}\right\}^{-1} . \tag{4}$$

The number of hydrogen ions produced is

$$n_{\text{H}} = 1 - \left\{\frac{(\text{H}^+)}{K_{1\text{ADP}}}\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgHADP}}}\right) + \frac{2(\text{H}^+)^2}{K_{1\text{ADP}}K_{2\text{ADP}}}\right\} f_{\text{ADP}} - \frac{(\text{H}^+)}{K_{\text{G6P}}} f_{\text{G6P}}$$
$$+ \left\{\frac{(\text{H}^+)}{K_{1\text{ATP}}}\left(1 + \frac{(\text{Mg}^{2+})}{K_{\text{MgHATP}}}\right) + \frac{2(\text{H}^+)^2}{K_{1\text{ATP}}K_{2\text{ATP}}}\right\} f_{\text{ATP}} \ . \tag{5}$$

In a buffered medium, the enthalpy change ($\Delta H^\circ_{\text{OBS}}$) is given by*

$$\Delta H^\circ_{\text{OBS}} = \Delta H^\circ_{\text{A}} + n_{\text{H}}\Delta H^\circ_{\text{C}} \ , \tag{6}$$

where $\Delta H^\circ_{\text{C}}$ refers to the enthalpy change for the buffer protonation process:

$$\text{TRIS} + \text{H}^+ = \text{TRIS}\cdot\text{H}^+ \ . \tag{C}$$

It should be noted that eqs. (1) to (5) are similar to Alberty's [2] eqs. (21) to (23) and (26), which apply to the hydrolysis reaction of ATP, with the exception of the addition of terms to account for the presence [6] of $\text{Mg}_2\text{ATP}$ and the various G6P species. As has been pointed out previously [2], in spite of its apparent complexity, the only function of eq. (1) is to weight appropriate enthalpies for specific ionic reactions properly in correct proportions to the actual ionic populations [given by eqs. (2) to (4)] present in a given system.

The equilibrium constant for (A) is given by

$$K_{\text{OBS}} = \frac{(\text{ADP})_{\text{T}}(\text{G6P})_{\text{T}}}{(\text{ATP})_{\text{T}}(\text{Glucose})_{\text{T}}} = \frac{K_{\text{B}}f_{\text{ATP}}}{f_{\text{ADP}}f_{\text{G6P}}(\text{H}^+)} , \tag{7}$$

where $(\text{ATP})_{\text{T}}$, $(\text{ADP})_{\text{T}}$, $(\text{Glucose})_{\text{T}}$, and $(\text{G6P})_{\text{T}}$ are, respectively, the total concentrations of ATP, ADP, Glucose, and G6P present in a given system and $K_{\text{B}}$ is the equilibrium constant for reaction (B). For the experiments reported herein, $K_{\text{OBS}}$ was computed to be sufficiently large that no corrections need be made for incomplete reaction.

### *3.2. Thermodynamic constants used in coupled equilibrium analysis*

The pK and enthalpy values used in our computation are given in table 2. The numerical values of $\text{p}K_{\text{G6P}}$ and $\Delta H^\circ_{\text{G6P}}$ given in table 2 are estimated from existing data for ribose 5-phosphate [8] and glucose 1-phosphate [9]; the estimated values for $\text{p}K_{\text{MgG6P}}$ and $\Delta H^\circ_{\text{MgG6P}}$ are based on data for the magnesium complex of glucose 1-phos-

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* The subscript OBS refers to an observable enthalpy of reaction which may either be calculated using eqs. (1) to (6) or measured.

phate [10]. The value given for $\Delta H^\circ_{\mathrm{Mg_2ATP}}$ is estimated using the selected values for $\mathrm{p}K_{\mathrm{Mg_2ATP}}$ and $\Delta S^\circ_{\mathrm{MgHATP}}$. A summary of pK values from the literature (thru 1965) for the other ATP and ADP species may be found in the review by Phillips [11]. The uncertainties given in table 1 take on a particular significance, in that they are subsequently to be used in order to estimate inaccuracies in other quantities.

### *3.3. Calculation of pMg and ionic strength*

The total concentration of magnesium ($(\mathrm{Mg})_\mathrm{T}$) present in a given solution is given by

$$(\mathrm{Mg})_\mathrm{T} = (\mathrm{Mg}^{2+}) + (\mathrm{MgADP}^{1-}) + (\mathrm{MgHADP}^\circ) + (\mathrm{MgG6P}^\circ) + (\mathrm{MgATP}^{2-}) + (\mathrm{MgHATP}^{1-}) + 2(\mathrm{Mg_2ATP}^\circ)\,. \quad (8$$

Making use of the appropriate equilibrium constants, it is a straight-forward procedure to show that

$$(\mathrm{Mg})_\mathrm{T} = (\mathrm{Mg}^{2+}) + \left(\frac{1}{K_{\mathrm{MgADP}}} + \frac{(\mathrm{H}^+)}{K_{\mathrm{MgHADP}}K_{\mathrm{1ADP}}}\right)(\mathrm{Mg}^{2+})\,f_{\mathrm{ADP}}(\mathrm{ADP})_\mathrm{T}$$

$$+ \left(\frac{1}{K_{\mathrm{MgATP}}} + \frac{(\mathrm{H}^+)}{K_{\mathrm{MgHATP}}K_{\mathrm{1ATP}}} + \frac{2(\mathrm{Mg}^{2+})}{K_{\mathrm{Mg_2ATP}}K_{\mathrm{MgATP}}}\right)(\mathrm{Mg}^{2+})\,f_{\mathrm{ATP}}(\mathrm{ATP})_\mathrm{T} + \frac{(\mathrm{Mg}^{2+})}{K_{\mathrm{MgG6P}}}\,f_{\mathrm{G6P}}(\mathrm{G6P})_\mathrm{T}\,. \quad (9$$

Hence, $(\mathrm{Mg}^{2+})$ may be solved for by an iterative calculation using eq. (9) and the experimentally known values o $(\mathrm{Mg})_\mathrm{T}$. The ionic strength is calculated using the concentrations of the relevant ions, which in turn are computed from the experimental concentrations and previously reported equilibrium constants (given in table 2). A complication is introduced in that many of these equilibrium constants are functions of ionic strength. Hence, a combin and iterative computation of both ionic strength and $(\mathrm{Mg}^{2+})$ was employed in this work, using initial guesses of t ionic strength to solve for $(\mathrm{Mg}^{2+})$, which is then used to recalculate the ionic strength in the next iteration. This cedure is continued until a self-consistent set of solutions for these equations is obtained.

In order to estimate uncertainties to be associated with the values of $\mathrm{pMg_{CALC}}$, the effects of variations due t the possible inaccuracies (given in table 2) in the assumed thermodynamic constants were calculated. The total estimate of uncertainty was then obtained by combining, in quadrature, the effects due to variations in the indiv parameters. The results of these calculations are the basis for the stated uncertainties given for the pMg values in table 1. Using a similar procedure, it was found that the computed ionic strength was insensitive to similar variations in the thermodynamic constants, i.e., the total estimate of uncertainty was less than 0.01 unit of ionic strei in all cases. The results of these calculations are given in table 1.

### *3.4. Comparison of calculations with measurements*

Given in table 1, and shown graphically in fig. 1, are values of $\Delta H_{\mathrm{OBS}}$ calculated using eqs. (1) to (6) and the thermodynamic constants from table 2. Also given in table 1 are uncertainties to be associated with the calculate values of $\Delta H_{\mathrm{OBS}}$ that were estimated by means of a scheme like that used in obtaining the uncertainties for $\mathrm{pMg_{CALC}}$. In fig. 2 the differences, $\Delta H_{\mathrm{OBS}}$(calculated) − $\Delta H_{\mathrm{OBS}}$(measured), are plotted as a function of $\mathrm{pMg_C}$ It is thus seen that within the indicated uncertainties, $\Delta H_{\mathrm{OBS}}$(calculated) agrees with $\Delta H_{\mathrm{OBS}}$(measured) except for the range pMg = 3.3 to 5.4, although the uncertainties become progressively larger as pMg decreases.

It should be noted that the calculated values of $\Delta H_{\mathrm{OBS}}$ show an almost total insensitivity to those thermodyi mic parameters involving protonation processes, namely: $\mathrm{p}K_{\mathrm{1ATP}}$, $\mathrm{p}K_{\mathrm{2ATP}}$, $\mathrm{p}K_{\mathrm{1ADP}}$, $\mathrm{p}K_{\mathrm{G6P}}$, $\mathrm{p}K_{\mathrm{MgHATP}}$, $\mathrm{p}K_{\mathrm{Mgl}}$ and the corresponding enthalpies. Hence, any lack of agreement between $\Delta H_{\mathrm{OBS}}$(calculated) and $\Delta H_{\mathrm{OBS}}$(meası assuming negligible errors in $\Delta H_{\mathrm{OBS}}$(measured), must be ascribed either to inaccuracies in the other thermodyna parameters, or, less likely, to some serious shortcoming in the coupled equilibrium analysis used herein. Since thı remain eight thermodynamic parameters ($\mathrm{p}K_{\mathrm{MgATP}}$, $\mathrm{p}K_{\mathrm{MgADP}}$, $\mathrm{p}K_{\mathrm{MgG6P}}$, $\mathrm{p}K_{\mathrm{Mg_2ATP}}$, and the corresponding enthalpies) wherein the inaccuracies responsible for any discrepencies may reside, it does not appear to be possible assign the inaccuracies to any of one of these parameters. Worthy of further inquiry, however, are the following:

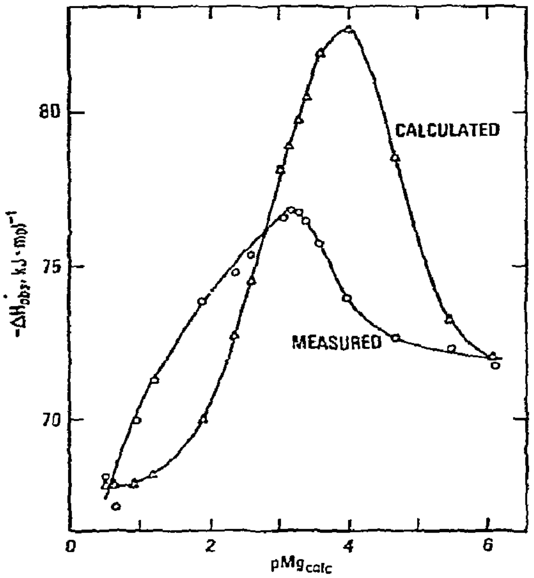

Fig. 1. Plot of $\Delta H_{OBS}$, measured (indicated by circles) and calculated (indicated by triangles), as a function of $pMg_{CALC}$.

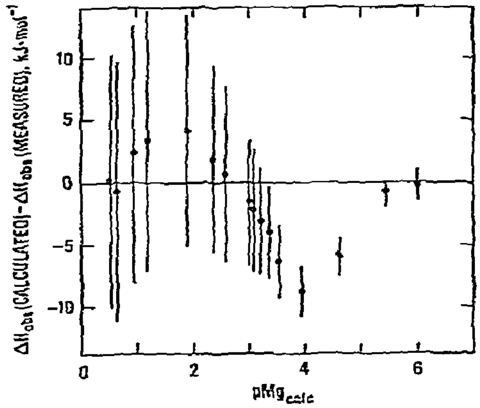

Fig. 2. Plot of the differences, [$\Delta H_{OBS}$(calculated) − $\Delta H_{OBS}$ (measured)], as a function of $pMg_{CALC}$. The length of a drawn bar is determined by the sum of the estimated uncertainties for the calculated and measured enthalpies.

the previous investigations that have led to values for $pK_{MgATP}$ and $\Delta H^{\circ}_{MgATP}$ do not appear to have taken into account the presence of the species $Mg_2ATP^{\circ}$, which has only recently been reported to exist in any meaningful quantities [6]; it was necessary to estimate values for $\Delta H^{\circ}_{Mg_2ATP}$, $pK_{MgG6P}$, and $\Delta H^{\circ}_{MgG6P}$; and most of the enthalpy data in table 2, with the exception of $\Delta H^{\circ}_{MgATP}$ and $\Delta H^{\circ}_{MgADP}$, were based on equilibrium constants determined at several temperatures. Calorimetric measurements of these quantities would be desirable.

### *3.5. Thermochemical implications*

Fig. 1 and eqs. (1) to (6) have important implications for the experimentalist. Thus, if an enthalpy of reaction corresponding to definite ionic species is desired, it is necessary either to perform measurements under very carefully selected conditions, or to be prepared to make the appropriate corrections. At $pMg \geqslant 6$, the measured enthalpy of reaction corresponds to the enthalpy change for process (*B*), while at $pMg \leqslant 0.5$, the measured enthalpy change, corrected for buffer protonation, corresponds to the process

$$\text{eq-D-glucose} + Mg_2ATP^{\circ} = \text{eq-D-}MgG6P^{\circ} + MgADP^{1-} + H^{+}\,, \qquad \text{(D)}$$

and is approximately equal to $-19.9$ kJ mol$^{-1}$.

Using our self-consistent procedure for calculating free magnesium ion concentration, we calculate, neglecting the effect of sodium ion binding to reactants and products, that under the experimental conditions used* by McGlothlin and Jordan [17], their enthalpy measurements were performed at $pMg = 1.61$ and $3.65$. They report a $\Delta H_{OBS} = -74.9 \pm 1.5$ kJ mol$^{-1}$ in TRIS/TRIS·HCl buffer (pH = 8.0, ionic strength = 0.3, $T = 25.0$°C) under both sets of conditions, finding no significant difference in their measured enthalpies within their experimental imprecision. Using the experimental enthalpy data reported herein (see fig. 1), we obtain $\Delta H_{OBS} = -75.3 \pm 0.3$

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* McGlothlin and Jordan used two different sets of experimental conditions. In one, ATP and magnesium chloride were present in the *mass* ratio 1 : 1, while in the second the *molar* ratio of these two materials was 1 : 1 [18].

kJ mol$^{-1}$ at pMg = 1.61 and $\Delta H_{OBS} = -72.8 \pm 0.3$ kJ mol$^{-1}$ at pMg = 3.65. The former value is in exceller ment with the value of McGlothlin and Jordan, while the latter value differs from their result by an amou lies only slightly outside the given uncertainty intervals.

## Acknowledgement

This research was supported in part by the Office of Standard Reference Materials of the National Bur Standards.

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