THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 261, No. 27, Issue of September 25, pp. 12733-12737, 1986
Printed in U.S.A.

Thermodynamics of the Hydrolysis of Adenosine 5′-triphosphate to Adenosine 5′-diphosphate*

(Received for publication, June 2, 1986)

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The enthalpy of hydrolysis of the enzyme-catalyzed (heavy meromyosin) conversion of adenosine 5′-triphosphate (ATP) to adenosine 5′-diphosphate (ADP) and inorganic phosphate has been investigated using heat-conduction microcalorimetry. Enthalpies of reaction were measured as a function of ionic strength (0.05–0.66 mol kg^{-1}), pH (6.4–8.8), and temperature (25–37 °C) in Tris/HCl buffer. The measured enthalpies were adjusted for the effects of proton ionization and metal ion binding, protonation and interaction with the Tris buffer, and ionic strength effects to obtain a value of $\Delta H^0 = -20.5 \pm 0.4$ kJ mol^{-1} at 25 °C for the process,

$$ATP^{4-}(aq) + H_2O(l) = ADP^{3-}(aq) + HPO_4^{2-}(aq) + H^+(aq)$$

where aq is aqueous and l is liquid. Heat measurements carried out at different temperatures lead to a value of $\Delta C_p^0 = -237 \pm 30$ J mol^{-1} K^{-1} for the above process.

The biochemical significance of an understanding of the thermodynamics of the hydrolysis of ATP was recognized over 50 years ago by Meyerhof and Lohmann (1). During the late 1960s, thorough reviews of this subject were prepared by Alberty (2–4) and by Phillips *et al.* (5). These reviews emphasized the need for accurate thermodynamic data for the following reference reaction,

$$ATP^{4-}(aq) + H_2O(l) = ADP^{3-}(aq) + HPO_4^{2-}(aq) + H^+(aq) \quad (A)$$

where aq is aqueous and l is liquid. Specifically, one desires a knowledge of the Gibbs energy, enthalpy, entropy, and heat capacity changes for the above process. This information, together with data on the proton and metal ion binding to reactants and products allows one to calculate the thermodynamic behavior of the overall hydrolysis reaction,

$$\Sigma ATP + H_2O(l) = \Sigma ADP + \Sigma P_i + n_H H^+ \quad (B)$$

as a function of pH, metal ion concentration, and temperature. The summation (Σ) in the above equation denotes that one is dealing with a multiplicity of ionic and metal-bound phosphate species. The Gibbs energy change for process A was the subject of an equilibrium investigation by Guynn and Veech (6) who also summarized earlier studies (7–9). The enthalpy change has been determined by Kitzinger and Benzinger (10) and by Podolsky *et al.* (11, 12). These calorimetric studies were carried out at high ionic strengths, probably since the enzyme used (myosin) is soluble only under these conditions. These studies did not systematically investigate the effects of variations of pH, ionic strength, or temperature on the enthalpy of reaction. Also, the results of two of the investigations (10, 12) differ from that of the third (11) by 3 kJ mol^{-1}. For these reasons and also since advances in calorimetric instrumentation now make possible more precise measurements, we have carried out this study. The primary goal herein is to obtain reliable values for the standard state enthalpy and heat capacity changes for Process A, the reference reaction for the hydrolysis of ATP. Combination of this data with the Gibbs energy change yields a value for the entropy change and thus a more complete understanding of the thermodynamics of this reaction. We also note the recent calorimetric measurements on the intermediate steps of ATP hydrolysis (13–15). These studies which seek a knowledge of the thermodynamics of the intermediate steps require, as base-line data, the magnitude of the thermodynamic changes for the overall hydrolysis process.

EXPERIMENTAL PROCEDURES

The heavy meromyosin (the ATPase active fragment from trypsin-digested myosin) and the disodium salt of ATP were obtained from Sigma.[1] The Tris, HCl, and $CaCl_2$ were obtained from Fisher Scientific. Information obtained from the vendor on the purity of the ATP sample is as follows: purity > 99% by TLC: no ADP or AMP detected; vanadium < 5 ppm; calcium < 30 ppm; iron < 9 ppm; magnesium < 4 ppm; inorganic phosphate < 0.03%; chloride < 0.04%; and moisture by Karl Fischer 8.8%. Chromatographic analysis (Synchropak AX100 weak anion exchange column with a 0.2 M ammonium phosphate mobile phase at pH 2.7) of the ATP sample indicated no impurities in the sample. Our own Karl Fischer analysis yielded a moisture content of 9.0% for the ATP sample. A correction for this moisture content was applied to all calorimetric measurements.

Chromatographic analysis of the reacted mixture of ATP in Tris/HCl using meromyosin at pH 8.0 and 298.15 K showed that the conversion of ATP to ADP and inorganic phosphate was complete. This is consistent with the available equilibrium data from the literature (2, 6) and is indicative of a pure sample of ATP. There was also no chromatographic evidence of any side reactions.

The microcalorimetric procedures have been described previously (16, 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 solid ATP in a Tris/HCl buffer containing $CaCl_2$. The enzyme solution was prepared by dialyzing the enzyme preparation received from the vendor against the buffer used to dissolve the ATP. A "blank" heat effect of 3.2 ± 1.0 mJ was measured when a substrate solution consisting of ADP in Tris/HCl buffer containing $CaCl_2$ was mixed with an enzyme solution containing meromyosin dialyzed against Tris/HCl buffer. The concentrations of the ADP, Tris/HCl, $CaCl_2$, and meromyosin were essentially identical to those used in the corresponding reaction heat measurements where the reactant ATP was used instead of the product ADP. This "blank" heat effect was applied as a correction (≈1%) to all of the measurements of heat of reaction.

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

TABLE I

Measured enthalpies of hydrolysis of ATP to ADP and inorganic phosphate

All measurements were performed in Tris/HCl buffer at the temperatures and pH values indicated. The total concentration of ATP was ~5.2 mmol (kg solution)$^{-1}$, and the concentration of free calcium ion was 1.0×10^{-5} mol liter^{-1}. All uncertainties are imprecisions and refer to 95% confidence limits. N is the number of measurements performed.

Series A, measurements at 298.15 K, Tris/HCl (0.1 mol liter^{-1})

pH	$-\Delta H$/kJ mol^{-1}	N	pH	$-\Delta H$/kJ mol^{-1}	N
6.42	54.88 ± 2.0	6	8.32	67.57 ± 0.17	6
7.16	59.40 ± 0.90	3	8.41	68.28 ± 0.16	3
7.55	65.32 ± 0.53	5	8.59	68.03 ± 0.37	6
7.90	65.87 ± 0.33	4	8.63	67.50 ± 0.19	6
			8.80	68.10 ± 0.23	8

Series B, measurements performed with varying concentrations of Tris/HCl buffer at 298.15 K

pH	Tris/HCl concentration/mol liter^{-1}	$-\Delta H$/kJ mol^{-1}	N
8.57	0.25	68.66 ± 0.28	6
8.53	0.60	70.23 ± 0.10	6
8.55	1.00	71.75 ± 0.70	4

Series C, measurements at 298.15 K. The 0.1 M Tris/HCl contained KCl (0.6 mol liter^{-1})

pH	$-\Delta H$/kJ mol^{-1}	N
7.86	67.07 ± 0.26	6
8.14	68.28 ± 0.16	6
8.19	68.17 ± 0.24	3
8.45	68.11 ± 0.36	3

Series D, measurements at higher temperatures. The buffer was 0.1 M Tris/HCl at pH 8.42

Temperature	$-\Delta H$/kJ mol^{-1}	N
K		
304.15	69.16 ± 0.20	6
309.95	70.25 ± 0.44	9

The rate of the hydrolysis reaction was found to be too slow to allow meaningful heat measurements to be performed when only the meromyosin was used. Thus, calcium chloride was added to the reacting solutions to activate the enzyme and to achieve a rapid hydrolysis reaction which was complete in less than 30 min. The average total concentration of $CaCl_2$ in the calorimetric measurements was 1.58 ± 0.03 mmol (kg solution)$^{-1}$ while the total ATP concentration was 5.20 ± 0.06 mmol (kg solution)$^{-1}$. The concentration of free calcium ion in the reacting solutions was found to be 1×10^{-5} mol liter^{-1} using a NOVA 8 ionized calcium analyzer which was calibrated using known concentrations of calcium chloride in Tris/HCl buffer.[2] These measurements were performed at the lower limit of sensitivity of the instrumentation. The value obtained should be considered to be the upper limit to the concentration of free calcium ion.

Measurement of pH was performed using recommended procedures (18). All reacting solutions were prepared gravimetrically using calibrated balances.

RESULTS AND DISCUSSION

A major objective of this investigation was the determination of the standard state enthalpy and heat capacity change for Process A. Thus, calorimetric measurements were performed which involved variations in pH, buffer concentration, and temperature. Also, since the earlier calorimetric investigations (10–12) used solutions containing 0.6 M KCl, it was deemed *desirable* to perform measurements under similar conditions for the purpose of comparison and also to determine the magnitude of ionic strength effects. The calorimetric results are summarized in Table I and shown in Figs. 1–4.

[2] This corresponds to $p\mathrm{Ca} = -\log_{10}[\mathrm{Ca}^{2+}] = 5.0$.

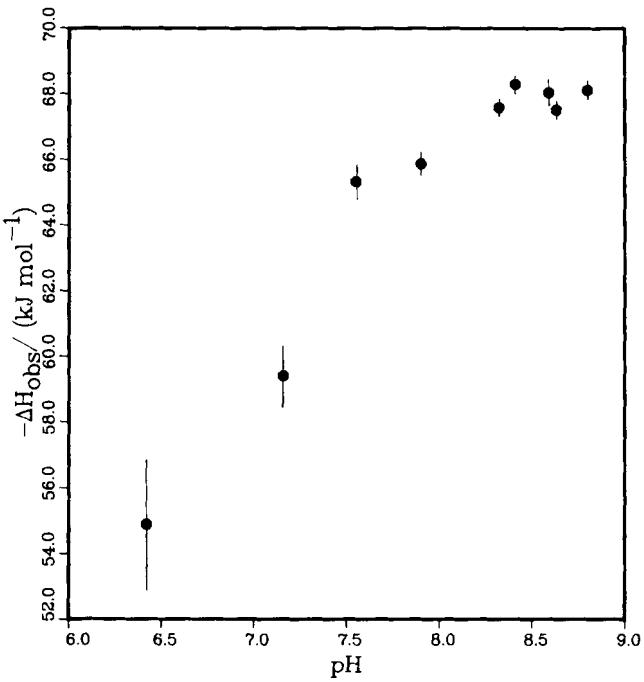

FIG. 1. **The enthalpy of hydrolysis of ATP as a function of pH at 298.15 K.** All measurements were carried out in 0.1 M Tris/HCl using ATP concentrations of 5.20 mmol (kg solution)$^{-1}$ and at a free calcium ion concentration of 1.0×10^{-5} mol liter^{-1}. The *error bars* shown correspond to 95% confidence limits.

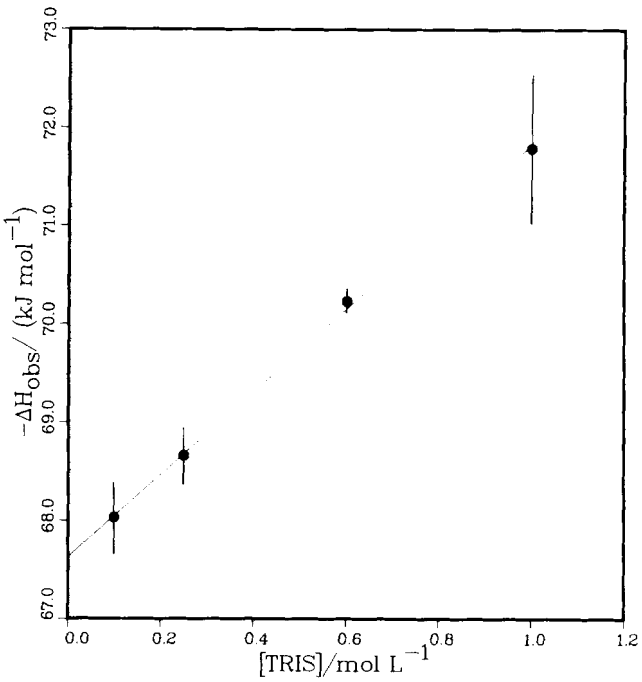

FIG. 2. **The enthalpy of hydrolysis of ATP at 298.15 K as a function of the concentration of the Tris buffer.** The pH was 8.56 ± 0.03 and the pCa was 5.0. The *straight line* is a least squares fit to the measurements and has an intercept of 67.63 ± 0.12 kJ mol^{-1} and a slope of 4.20 ± 0.10 kJ mol^{-1} liter. The *error bars* correspond to 95% confidence limits.

The interpretation of the measurements is done in the context of the equations of Alberty (2–4) except that the effects of ionic strength are also made a part of the calculation by the inclusion of an extended Debye-Hückel expression for

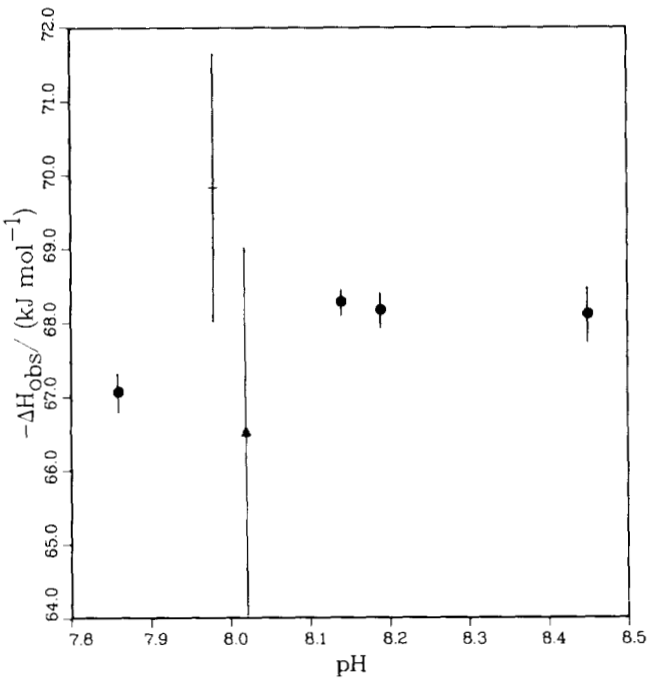

FIG. 3. **The enthalpy of hydrolysis of ATP as a function of pH at 298.15 K carried out in 0.1 M Tris/HCl buffer containing 0.6 M KCl.** The data sets shown are our own (●), Podolsky and Sturtevant (▲) (11), and the result (+) of Kitzinger and Benzinger (10) and of Podolsky and Morales (12) adjusted to 298.15 K.

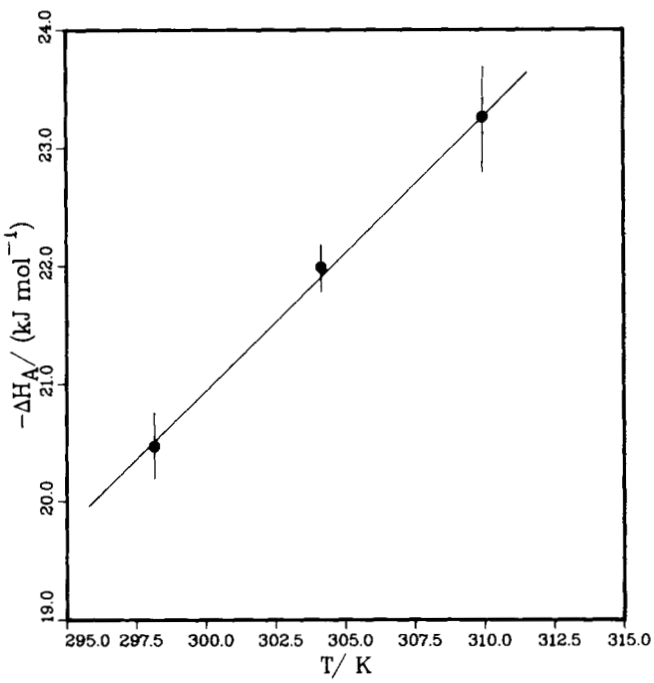

FIG. 4. **The enthalpy of hydrolysis of ATP as a function of temperature.** All measurements were carried out at pH 8.41 ± 0.01 in 0.1 M Tris/HCl buffer. The data shown in this figure were corrected for the enthalpy of protonation of the Tris buffer and for ionization and metal ion binding effects. The *error bars* correspond to 95% confidence limits. The slope of the curve yields a value of $\Delta C_p^0 = -237 \pm 30$ J mol^{-1} K^{-1}.

the activity coefficients.

$$\ln \hat{\gamma}_i = -A_m z_i^2 \hat{I}^{1/2}/(1 + B\hat{I}^{1/2}) \quad (1)$$

In the above equation $\hat{\gamma}_i$ and z_i are, respectively, the activity coefficient and the charge of the *ith* species, A_m is a Debye-Hückel constant (19), B is an "ion-size" parameter, and $\hat{I}$ is the ionic strength which is calculated as follows.

$$\hat{I} = (1/2)\ \Sigma \hat{m}_i z_i^2 \quad (2)$$

The above summation includes the molalities ($\hat{m}_i$) of all of the species in the solution. The symbol "^" denotes a quantity which pertains to a species as distinct from a stoichiometric quantity (20). The activity coefficients provide an adjustment to the Gibbs energies or equilibrium constants for the effects of nonideality due to long-range electrostatic forces. Thus the excess Gibbs energy of the *ith* species is given by,

$$\hat{G}_i^{ex} = RT \ln \hat{\gamma}_i \quad (3)$$

where R and T are, respectively, the gas constant (8.31441 J mol^{-1} K^{-1}) and the thermodynamic temperature. The temperature derivative of the excess Gibbs energy yields an expression for the excess enthalpy,

$$\hat{H}_i^{ex} = (1/2)A_L z_i^2 \hat{I}^{1/2}/(1 + B\hat{I}^{1/2}) \quad (4)$$

where A_L is the Debye-Hückel constant for the enthalpy. Equation 4 was used to adjust enthalpies of reaction to the ionic strength required for the calculations. In performing the equilibrium calculations a value of 1.6 was assumed for the B parameter in Equations 1 and 4. All calculations were made self-consistent in regard to the ionic strength (20).

The thermodynamic data relevant to the analysis of the results are summarized in Table II. The notation used to denote the various processes follows Alberty's earlier usage (2–4). The Gibbs energy change for Process A given in Table II is the average of the results of Benzinger *et al.* (7) and of Guynn and Veech (6) adjusted to 298.15 K and to the hypothetical one molal standard state for all reactants and products. The enthalpy and heat capacity changes for Process A given in Table II are the final results obtained in this study.

The numerical solution of the equilibrium equations involves the consideration of the 12 equilibria shown in Table II and up to 21 different chemical species. The results of these calculations yield concentrations of all of the species in solution under a given set of conditions, the ionic strength of the solution, the number of protons produced (n_H), and values of the observed equilibrium constant (K_{obs}) and the observed enthalpy change (ΔH_{obs}) for the overall process occurring in aqueous solution (see Process A). The observed equilibrium constant is given by (3):

$$K_{obs} = [\Sigma ADP][\Sigma P_i]/[\Sigma ATP] = K_A f_{ATP}/(f_{ADP} f_{P_i}[H^+]). \quad (5)$$

In the above equation f_i is the fraction of the *ith* species existing in the most basic form (*e.g.* ATP^{4-}, ADP^{3-}, and HPO_4^{2-}). The observed enthalpy is given by (4):

$$\Delta H_{obs} = \Delta H_A + \Delta H' + n_H \Delta H_C \quad (6)$$

where ΔH_C is the enthalpy change for the buffer protonation reaction:

$$\text{Tris(aq)} + H^+(aq) = \text{Tris}\cdot H^+(aq) \quad (C)$$

The term $\Delta H'$ is the contribution to the enthalpy change due to proton ionization and metal ion binding. It is identical to the three terms on the right side of Alberty's (4) equation (28) with the exception that "Ca" replaces "Mg" in all places.

Examination of the experimental data (see Table I) shows the following. 1) ΔH is essentially constant between pH 8.32 and 8.80 in series A. The average is -67.86 ± 0.22 kJ mol^{-1}. 2) The average result obtained in series C between pH 8.14 and 8.45 is also constant and is -68.20 ± 0.17 kJ mol^{-1}. This value is only slightly more exothermic (0.34 kJ mol^{-1}) than

TABLE II

Selected values of thermodynamic constants relevant to the hydrolysis of ATP

All reactions pertain to aqueous solutions at 298.15 K. The ionic strength to which the value pertains is given for both the Gibbs energy change or pK and for the enthalpy change, but not for the heat capacity change.

Process	pK or ΔG^0	I	ΔH^0	I	ΔC_p^0	References
		mol kg^{-1}	*kJ mol^{-1}*	*mol kg^{-1}*	*J mol^{-1} K^{-1}*	
$ATP^{4-} + H_2O = ADP^{3-} + HPO_4^{2-} + H^+$	$\Delta G_A^0 = 3.4$ kJ mol^{-1}	0.0	−20.5 ± 0.4	0.0	−237 ± 30	This work and 6, 7
$HATP^{3-} = H^+ + ATP^{4-}$	p$K_{1ATP} = 6.95$	0.2	−7.03	0.2	−126[a]	3
$H_2ATP^{2-} = H^+ + HATP^{3-}$	p$K_{2ATP} = 4.06$	0.2	0.0	0.2	−63[a]	3
$HADP^{2-} = H^+ + ADP^{3-}$	p$K_{1ADP} = 6.88$	0.2	−5.73	0.2	−126[a]	3
$H_2ADP^- = H^+ + HADP^{2-}$	p$K_{2ADP} = 3.93$	0.2	4.18	0.2	−63[a]	3
$H_2PO_4^- = H^+ + HPO_4^{2-}$	p$K_{2P} = 7.20$	0.0	4.14	0.0	−226	23
$CaATP^{2-} = Ca^{2+} + ATP^{4-}$	p$K_{CaATP} = 3.96$	0.1	3.8	0.1	−126[a]	24
$CaHATP^- = Ca^{2+} + HATP^{3-}$	p$K_{CaHATP} = 2.13$	0.1	1.26	0.1	−188[a]	26
$CaADP^- = Ca^{2+} + ADP^{3-}$	p$K_{CaADP} = 2.86$	0.1	5.0	0.1	−188[a]	25
$CaHADP^0 = Ca^{2+} + HADP^{2-}$	p$K_{CaHADP} = 1.58$	0.1	2.5	0.1	−251[a]	25
$CaHPO_4^0 = Ca^{2+} + HPO_4^{2-}$	p$K_{CaP} = 2.40$	0.0	−10.0	0.0	−300[b]	26
$Tris \cdot H^+ = Tris^0 + H^+$	p$K_{Tris} = 8.072$	0.0	47.48	0.0	−50	23, 27, 28, 29

[a] Estimated. Also see Ref. 4.

[b] Estimated. The value of ΔC_p^0 calculated from the data of Gregory *et al.* (26) is excessively large.

the value obtained in the absence of KCl. The difference is within the random error of the measurements. 3) The measurements performed in varying concentrations of Tris buffer (series B) lie on a straight line having a slope of 4.20 kJ mol^{-2} liter and an intercept of −67.63 kJ mol^{-1}.

From the equilibrium modeling calculations it was found that: 1) Ionic strength effects cannot explain the magnitude of the variation observed in the measured enthalpies with the concentration of Tris, and 2) the calculated difference in the enthalpies due to the presence of 0.6 M KCl is 0.15 kJ mol^{-1}. This is in agreement with the measured difference of 0.34 ± 0.28 kJ mol^{-1}.

Thus, on the basis of both the experimental results and the equilibrium modeling calculations we conclude that there is a specific interaction between the Tris buffer and one or more of the reactants and products that is not accounted for in the equilibrium model of the system. Therefore, the experimental data in series C were extrapolated to zero buffer concentration (see Fig. 2) to eliminate this effect. Thus, $\Delta H_{obs} = -67.63$ kJ mol^{-1} at pH 8.57 at an ionic strength of 0.055 mol kg^{-1} and at 298.15 K in the absence of this buffer effect. To obtain the desired value of ΔH_A^0, corrections must still be applied for proton ionization and metal ion binding effects, for protonation of the buffer (see Equation 6), and for ionic strength effects. Therefore, using the data in Table II and the equilibrium model, the following results are obtained for pH 8.57 and an ionic strength of 0.055 mol kg^{-1} at 298.15 K: $\Delta H' = +0.071$ kJ mol^{-1} and $n_H = 0.990$. The adjustment to zero ionic strength is estimated to be −0.25 kJ mol^{-1}.

Rather than rely solely upon the calculated value of n_H we use a value of n_H equal to unity. We do this based upon the measured value of 1.00 obtained by Podolsky and Morales (12) at pH 8.0 in both 0.05 and 0.60 M KCl solutions. Also, the measured enthalpies were found to be essentially constant from pH 8.31 to 8.80 (see Fig. 1). Application of a buffer protonation correction of +47.48 kJ mol^{-1}, a metal-ion and proton ionization correction of −0.071 kJ mol^{-1}, and an ionic strength correction of −0.25 kJ mol^{-1} to the value of −67.63 kJ mol^{-1} yields a value of $\Delta H_A^0 = -20.47$ kJ mol^{-1} at 298.15 K.

Similar treatment of the data obtained at 304.15 and 309.95 K (see series D in Table I) leads to values of $\Delta H_A^0 = -21.99$ and −23.26 kJ mol^{-1} at these respective temperatures. In performing these calculations, the equilibrium constants and

TABLE III

Judgment of possible sources of systematic errors associated with the measurement of the enthalpy change for Process A

Source of error	Estimate of error
	kJ mol^{-1}
Heat measurement error	0.14
Impurities in ATP	0.07
Moisture correction	0.20
Ionization and metal ion binding correction	0.10
Buffer protonation correction	0.25
Adjustment to standard state	0.10
Incomplete reaction	Negligible
Square root of total in quadrature	0.37

Gibbs energy changes in Table II were adjusted from 298.15 K to the higher temperatures using the following equation (21).

$$R \ln K = -\Delta G^0_{298.15}/298.15 + \Delta H^0_{298.15}((1/298.15) - (1/T)) + \Delta C_p^0((298.15/T) - 1 + \ln(T/298.15)) \quad (7)$$

Enthalpies were adjusted from 298.15 K to higher temperatures using

$$\Delta H^0_T = \Delta H^0_{298.15} + \Delta C_p^0(T - 298.15). \quad (8)$$

In using Equations 7 and 8, the heat capacity changes were assumed to be constant over the temperature range of interest. From the temperature dependence of ΔH_A^0 (see Fig. 4), a value of $\Delta C_p^0 = -237 \pm 30$ J mol^{-1} K^{-1} was calculated for Process A. The uncertainty is based solely upon random error estimates and refers to the 95% confidence limit.

Estimates of systematic error associated with the measurement of ΔH_A^0 are given in Table III. The most substantial errors are judged to lie in the moisture determination performed on the ATP sample and in the buffer protonation correction. The total estimate of systematic error (0.4 kJ mol^{-1}) is about 50% larger than the random error in the heat measurements. We prefer the error estimate based upon consideration of possible sources of systematic error and, therefore, adopt a final value of $\Delta H_A^0 = -20.5 \pm 0.4$ kJ mol^{-1} at 298.15 K. Since the systematic errors should be essentially independent of temperature, the estimate of error assigned to ΔC_p^0 is based only upon considerations of random error. Thus, $\Delta C_p^0 = -237 \pm 30$ J mol^{-1} K^{-1} for Process A over the temperature range 298–310 K. Combination of the value of ΔH_A^0

obtained herein with the value of the Gibbs energy change of 3.4 kJ mol^{-1} given in Table II yields a value of −80 J mol^{-1} K^{-1} for the standard state entropy change for Process A.

The interpretation of the early investigations of Meyerhof and Lohmann (1) and of Ohlmeyer (22) is complicated by a lack of consideration of the heat effects associated with buffer protonation, and it does not appear to be possible to make any meaningful comparison between their results and our own. The primary result of the studies of Kitzinger and Benzinger (10) and of Pokolsky and Morales (12) is based upon the same set of data obtained at 293 K in 0.10 M Tris/HCl containing 0.6 M KCl at pH 8.0 both with and without added $CaCl_2$ (1 mM). Under these conditions the measured enthalpy change was −68.5 ± 1.8 kJ mol^{-1}. Using the thermodynamic data in Table II we adjust this result to 298.15 K and obtain $\Delta H = -69.8 \pm 1.8$ kJ mol^{-1}. The result of Podolsky and Sturtevant (11) was obtained under similar conditions as used by the other workers (10, 12) except that the temperature was 298.15. They (11) obtained a value of −66.5 ± 2.5 kJ mol^{-1}. Comparison of these results with our own measurements performed in 0.6 M KCl is shown in Fig. 3. The difference between the earlier calorimetric data sets (10–12) is 3.3 kJ mol^{-1}. While this difference is 5% of the total measured heat effect in the Tris/HCl buffer, the difference becomes a much larger percentage (16%) after correction for the heat of protonation of the buffer. It is seen, however, that there is agreement between the different sets of measurements within the indicated limits of error.

There is no experimental data in the literature on the heat capacity change for Process A. Alberty (4), however, estimated a value of −60 cal mol^{-1} K^{-1} = −251 J mol^{-1} K^{-1} which is in excellent agreement with the value of −237 ± 30 J mol^{-1} K^{-1} obtained herein.

Acknowledgments—We thank Dr. Yadu Tewari for his chromatographic analysis of the ATP sample and of the final reaction mixture and Dr. Salvador Sena for measuring the concentration of free calcium ion.

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