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J. Chem. Thermodynamics 35 (2003) 1809–1830
JCT
www.elsevier.com/locate/jct

Thermodynamics of the hydrolysis reactions of adenosine 3′,5′-(cyclic)phosphate(aq) and phospho*enol*pyruvate(aq); the standard molar formation properties of 3′,5′-(cyclic)phosphate(aq) and phospho*enol*pyruvate(aq)

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Received 24 June 2003; accepted 6 August 2003

Abstract

Molar calorimetric enthalpy changes $\Delta_r H_m$(cal) have been measured for the biochemical reactions {cAMP(aq) + H_2O(l) = AMP(aq)} and {PEP(aq) + H_2O(l) = pyruvate(aq) + phosphate(aq)}. The reactions were catalyzed, respectively, by phosphodiesterase 3′,5′-cyclic nucleotide and by alkaline phosphatase. The results were analyzed by using a chemical equilibrium model to obtain values of standard molar enthalpies of reaction $\Delta_r H_m^\circ$ for the respective reference reactions {$cAMP^-$(aq) + H_2O(l) = $HAMP^-$(aq)} and {PEP^{3-}(aq) + H_2O(l) = $pyruvate^-$(aq) + HPO_4^{2-}(aq)}. Literature values of the apparent equilibrium constants K' for the reactions {ATP(aq) = cAMP(aq) + pyrophosphate(aq)}, {ATP(aq) + pyruvate(aq) = ADP(aq) + PEP(aq)}, and {ATP(aq) + pyruvate(aq) + phosphate(aq) = AMP(aq) + PEP(aq) + pyrophosphate(aq)} were also analyzed by using the chemical equilibrium model. These

Abbreviations: ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; cAMP, adenosine 3′,5′-(cyclic)phosphate; PEP, phospho*enol*pyruvate; GlyGly, glycylglycine; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); TAPS, *N*-[tris(hydroxymethyl)methyl-3-amino]propanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine; TEA, triethanolamine; Tris, 2-amino-2-hydroxymethylpropane-1,3 diol.

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0021-9614/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.jct.2003.08.002

calculations yielded values of the equilibrium constants K and standard molar Gibbs free energy changes $\Delta_r G^\circ_m$ for ionic reference reactions that correspond to the overall biochemical reactions. Combination of the standard molar reaction property values (K, $\Delta_r H^\circ_m$, and $\Delta_r G^\circ_m$) with the standard molar formation properties of the AMP, ADP, ATP, pyrophosphate, and pyruvate species led to values of the standard molar enthalpy $\Delta_f H^\circ_m$ and Gibbs free energy of formation $\Delta_f G^\circ_m$ and the standard partial molar entropy S°_m of the cAMP and PEP species. The thermochemical network appears to be reasonably well reinforced and thus lends some confidence to the accuracy of the calculated property values of the variety of species involved in the several reactions considered herein.
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Keywords: Adenosine 3′,5′-(cyclic)phosphate; Alkaline phosphatase; Calorimetry; Chemical thermodynamics; Enthalpy; Entropy; Gibbs free energy; Phosphodiesterase 3′,5′-cyclic nucleotide; Phospho*enol*pyruvate

1. Introduction

This study reports results of experiments and calculations that lead to the thermodynamic properties of adenosine 3′,5′-(cyclic)phosphate and phospho*enol*pyruvate (see figure 1) that play important roles in biochemistry and in physiology. Adenosine 3′,5′-(cyclic)phosphate or cAMP, is frequently referred to as the "second messenger". It is synthesized from ATP by the catalytic action of adenylate cyclase and its breakdown to AMP is brought about by the action of a phosphodiesterase that has specificity for cAMP. The two respective biochemical reactions are:

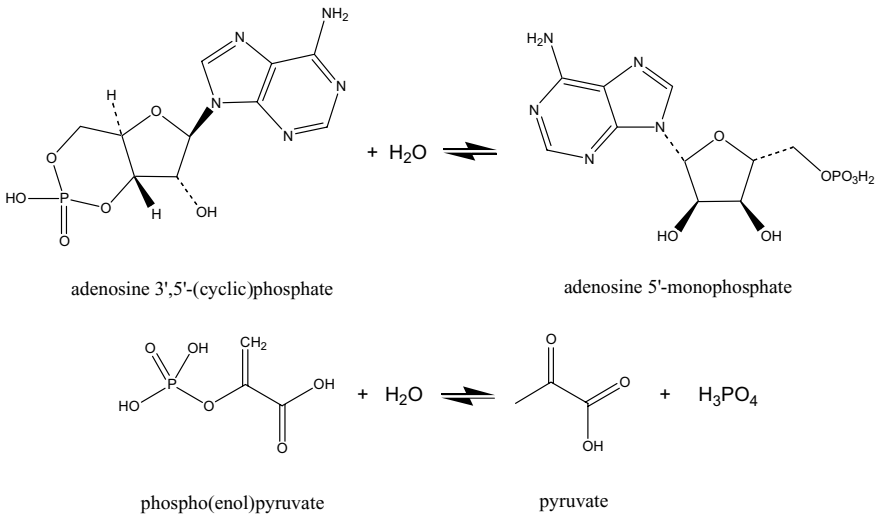

FIGURE 1. Structures of the substances in reactions (1) and (2). The neutral forms of the species are shown.

$$\mathrm{ATP(aq)} = \mathrm{cAMP(aq)} + \mathrm{pyrophosphate(aq)}, \quad (1)$$

$$\mathrm{cAMP(aq)} + \mathrm{H_2O(l)} = \mathrm{AMP(aq)}. \quad (2)$$

The physiological importance of cAMP arises from the fact that hormones work at cell surfaces to stimulate adenylate cyclase. This serves to increase the concentration of cAMP which then acts inside the cell as the intracellular mediator of the hormonal message (i.e., as the "second messenger"). The physiological effects of cAMP are, in general, exerted through the activation of specific protein kinases. Thus, cAMP is an essential element of control in many biological processes [1,2]. Several toxins operate by disrupting the delicate balance(s) required for the proper operation of cAMP.

Phospho*enol*pyruvate or PEP has often been referred to as a "high-energy" compound because of the very negative standard molar transformed Gibbs free energy change $\Delta_r G'^{\circ}_m$ for the hydrolysis reaction:

$$\mathrm{PEP(aq)} + \mathrm{H_2O(l)} = \mathrm{pyruvate(aq)} + \mathrm{phosphate(aq)}. \quad (3)$$

The PEP is formed during the breakdown of glucose to lactic acid. It then donates a phosphate to ADP by means of the following reaction:

$$\mathrm{PEP(aq)} + \mathrm{ADP(aq)} = \mathrm{pyruvate(aq)} + \mathrm{ATP(aq)}. \quad (4)$$

The PEP plays similar and important energy transfer roles in many biochemical processes, e.g., the Hatch–Slack (C_4) pathway for CO_2 fixation in plants, in the synthesis of the cell-walls of bacteria, in the initiation of the chorismate metabolic pathway, and in the operation of the heart [1,2].

In this study, we have used calorimetry to determine molar enthalpy changes for reactions (2) and (3). These results are then used in a chemical equilibrium model [3,4] to calculate standard molar enthalpy changes $\Delta_r H^{\circ}_m$ for chemical reference reactions that correspond to the overall biochemical reactions (2) and (3). These results, together with equilibrium data and formation properties from the literature, are then used to calculate the standard molar formation properties of the cAMP and PEP aqueous species. It will be seen that the results form a reasonably well reinforced thermochemical network that lends confidence to the accuracy of the calculated property values.

2. Experimental

2.1. Materials

Pertinent information on the substances used in this study is given in table 1. [1] The mass fraction of water in each of these samples was determined by means of Karl Fischer titration [5]. The sample of PEP had been characterized by high-perfor-

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

TABLE 1
Principal substances used in this study with their Chemical Abstracts Service (CAS) registry numbers, empirical formulae, relative molecular masses M_r, mass fraction moisture contents w determined by Karl Fischer analysis, suppliers (A, Aldrich; M, Mallinckrodt; and S, Sigma), estimated mole fraction purities x and method(s) used to determine x

Substance	CAS No.	Formula	M_r	w	Supplier	x	Method(s)
Adenosine 3′, 5′-(cyclic)phosphate, sodium salt	60-92-4	$C_{10}H_{11}N_5NaO_6P$	351.188	0.078	S	≈0.994	h.p.l.c.
Adenosine 5′-monophosphate, sodium salt	149022-20-8	$C_{10}H_{13}N_5NaO_7P$	369.203		S		
Alkaline phosphatase[a]			$4.697 \cdot 10^4$		S		h.p.l.c.
Imidazole	288-32-4	$C_3H_4N_2$	68.077		A		
Magnesium chloride	7786-30-3	$MgCl_2$	95.210		S		
Phosphodiesterase 3′, 5′-cyclic nucleotide[b]	9040-59-9		$1.714 \cdot 10^4$		S		
Phospho*enol*pyruvate, potassium salt	4267-07-0	$C_3H_4KO_6P$	206.132	0.014	S	≈0.992	
Phosphoric acid	7664-38-2	H_3PO_4	97.995		M		
Potassium phosphate, dibasic	7758-11-4	K_2HPO_4	174.16		S		
Pyruvic acid	127-17-3	$C_3H_4O_3$	88.062		S		

The estimated mole fraction purities are exclusive of the amounts of water in the samples. The h.p.l.c. methods used in our laboratory are described in the text (see Section 2.2).

[a] Lyophilized powder; EC number 3.1.3.1.

[b] Lyophilized powder; EC number 3.1.4.17.

mance liquid chromatography (h.p.l.c.) (u.v. detector) in an earlier study [5]; the finding was that the mole fraction x(impurities) was <0.01. We also examined the purity of the PEP by using an alternative h.p.l.c. method (see Section 2.2.2 below) and in this case x(impurities) was found to be <0.005. Similarly we used two different h.p.l.c. methods (see Section 2.2 below) to examine the purity of the cAMP: method 1 gave x(impurities) <0.007 and method 2 gave x(impurities) <0.005. The cAMP and PEP were stored at $T \approx 250$ K until ready for use.

2.2. Chromatography

2.2.1. H.p.l.c. method 1

The separation of the cAMP and AMP was done with an Agilent 1100 h.p.l.c. equipped with a u.v. detector set at the wavelength $\lambda = 260$ nm and an Alltech Inertsil ODS-2 column (4.6 mm i.d., 150 mm long) thermostatted at $T = 308$ K. The mobile phase was a gradient made from (I) {tetra butylammonium bromide (concentration $c = 0.005$ mol·dm^{-3}) + KH_2PO_4 ($c = 20$ mol·dm^{-3}), pH = 3.5} and (II) a solution that consisted of {solution (I) at the volume fraction $\phi(\mathrm{I}) = 0.40$ + acetonitrile at $\phi = 0.60$}. The following gradient of these two mobile phases was formed: $\phi(\mathrm{I}) = 0.90$ and $\phi(\mathrm{II}) = 0.10$ at time $t = 0$; and $\phi(\mathrm{I}) = 0.20$ and $\phi(\mathrm{II}) = 0.80$ at $t = 25$ min. The flow rate was 0.0167 cm^3·s^{-1}. The approximate retention times were 5.9 min and 9.3 min for AMP and cAMP, respectively. The limit of detection of cAMP was found to be $\approx 6 \cdot 10^{-7}$ mol·kg^{-1}.

2.2.2. H.p.l.c. method 2

The separation of PEP from phosphate was done with a Dionex DX 500 Ion Chromatograph with an ED50 conductivity detector (cell set at $T = 308$ K; temperature compensation = 1.7 K^{-1}; self-regenerating suppressor current $i = 300$ mA) and a Dionex AS11 anion exchange separation column (4 mm i.d., 250 mm long) with an AG11 guard column (4 mm i.d., 50 mm long). Both the column and the guard column were thermostatted in an LC25 chromatography oven at $T = 308$ K. The mobile phase consisted of (III) water and (IV) KOH in water ($c = 0.10$ mol·dm^{-3}). The KOH was generated by a Dionex EG-40 eluant generator cartridge. The following step-gradient of these two mobile phases was formed: $\phi(\mathrm{III}) = 0.90$ and $\phi(\mathrm{IV}) = 0.10$ at $t = 0$ min; $\phi(\mathrm{III}) = 0.90$ and $\phi(\mathrm{IV}) = 0.10$ at $t = 1.0$ min; $\phi(\mathrm{III}) = 0.60$ and $\phi(\mathrm{IV}) = 0.40$ at $t = 10.0$ min; $\phi(\mathrm{III}) = 0.60$ and $\phi(\mathrm{IV}) = 0.40$ at $t = 14.0$ min; $\phi(\mathrm{III}) = 0.10$ and $\phi(\mathrm{IV}) = 0.90$ at $t = 17.0$ min; $\phi(\mathrm{III}) = 0.10$ and $\phi(\mathrm{IV}) = 0.90$ at $t = 20.0$ min. The flow rate was 0.0167 cm^3·s^{-1}. The approximate retention times were 7.8 min and 10.2 min for phosphate and pyrophosphate, respectively. The limit of detection of PEP was found to be $\approx 2 \cdot 10^{-6}$ mol·kg^{-1}.

2.3. Degradation studies

Since the possibility of degradation of cAMP and PEP can affect the accuracy of the calorimetric results, studies of the instabilities of these two substances were performed by dissolving these two substances in the respective buffers used in the

calorimetric experiments and allowing these solutions to equilibrate at $T = 298.15$ K. The changes with time of their respective chromatographic peak areas were determined by using the h.p.l.c. methods described above. It was found that over a period of 2 h, the mole fraction changes with time for both substances were zero within the experimental error ($\pm 0.002 \cdot x$ for cAMP and $\pm 0.005 \cdot x$ for PEP).

2.4. Extent of reaction measurements

Attempts were made to measure the apparent equilibrium constants K' [6] of reactions (2) and (3). These studies used h.p.l.c methods 1 and 2, respectively. For reaction (2), the solution used for the forward direction of reaction consisted of [cAMP ($m = 0.0049$ mol·kg^{-1}) in phosphate buffer {$m(K_2HPO_4) = 0.101$ mol·kg^{-1} + $m(H_3PO_4) = 0.0138$ mol·kg^{-1}} + imidazole ($m = 5.2 \cdot 10^{-4}$ mol·kg^{-1}) + $MgCl_2$ ($m = 0.00107$) at pH = 7.53]. The reaction mixture used for the reverse direction of reaction consisted of [AMP ($m = 0.0050$ mol·kg^{-1}) in phosphate buffer {$m(K_2HPO_4) = 0.101$ mol·kg^{-1} + $m(H_3PO_4) = 0.0138$ mol·kg^{-1}} + imidazole ($m = 5.2 \cdot 10^{-4}$ mol·kg^{-1}) + $MgCl_2$ ($m = 0.00107$) at pH = 7.53]. The mass fraction of phosphodiesterase 3′,5′-cyclic nucleotide in the respective reaction mixtures was ≈0.0014. The forward and reverse reactions were allowed to proceed with gentle lateral shaking (≈30 shakes min^{-1}) at $T = 298.15$ K for 1 h and 4 h, respectively. For reaction (3), the solution used for the forward direction of reaction consisted of [PEP ($m = 0.0042$ mol·kg^{-1}) in phosphate buffer {$m(K_2HPO_4) = 0.102$ mol·kg^{-1} + $m(H_3PO_4) = 0.0141$ mol·kg^{-1}} at pH = 7.62]. The reaction mixture used for the reverse direction of reaction consisted of [pyruvic acid ($m = 0.0089$ mol·kg^{-1}) in phosphate buffer {$m(K_2HPO_4) = 0.102$ mol·kg^{-1} + $m(H_3PO_4) = 0.0141$ mol·kg^{-1}} at pH = 7.62]. The mass fraction of alkaline phosphatase in the respective reaction mixtures was $\approx 4 \cdot 10^{-4}$. The forward and reverse reactions were allowed to proceed with gentle lateral shaking (≈30 shakes min^{-1}) at $T = 298.15$ K for 1 h and 4 h, respectively.

2.5. Calorimetry

Descriptions of the microcalorimeters used in this study and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results have been given by Steckler *et al.* [7,8]. These calorimeters were calibrated electrically by using a high stability d.c. power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. The electric potential differences U of the thermopiles in the microcalorimeters are measured with Agilent model 34420A Nanovolt Meters. The values of U are then recorded on a microcomputer and the areas of the thermograms are calculated by numerical integration.

The calorimetric sample vessels were fabricated from high-density polyethylene. Each vessel had two compartments that held, respectively, ≈0.55 cm^3 and ≈0.40 cm^3 of solution. The substrate solutions were placed in the 0.55 cm^3 compartment and the enzyme solutions were placed in the 0.40 cm^3 compartment. The substrate solution contained either cAMP or PEP in a phosphate buffer. In the case of cAMP, the

buffer also contained the cofactors $MgCl_2$ and imidazole which were needed to make the hydrolysis reaction occur with sufficient rapidity for a precise calorimetric measurement. The respective enzyme solutions consisted of phosphodiesterase 3′,5′-cyclic nucleotide and alkaline phosphatase in the same phosphate buffer used to prepare the substrate solution.

The vessels and their contents were allowed to thermally equilibrate in the microcalorimeters for ≈60 min before the enzyme and substrate solutions were mixed. After mixing, ≈40 min was allowed for reaction (2) and ≈22 min for reaction (3). Following reaction, the vessels were removed from the microcalorimeters and chromatographic methods 1 and 2 (see Section 2.2) were used for the analysis of the final solutions from reactions (2) and (3), respectively. In all cases, it was found that the mole fraction of unreacted cAMP was $\leqslant 5 \cdot 10^{-4}$; small corrections to the amount of reacted cAMP were made for incomplete reaction. In the case of reaction (3), the chromatograms showed that, in all cases, no PEP remained. Based on the limit of detection of PEP, the mole fraction of unreacted PEP was $\leqslant 7 \cdot 10^{-4}$.

"Blank" enthalpy changes $\Delta_{mix}H$ were determined in control experiments. Thus, for reaction (2), $\Delta_{mix}H$ was $-(1.0 \pm 0.1)$ mJ for the mixing of the substrate solution with the buffer and $\Delta_{mix}H$ was $-(0.5 \pm 0.1)$ mJ for the mixing of the enzyme solution with the buffer. For reaction (3), $\Delta_{mix}H$ was (0.1 ± 0.7) mJ for the mixing of the substrate solution with the buffer and $\Delta_{mix}H$ was (0.1 ± 0.4) mJ for the mixing of the enzyme solution with the buffer. We judge the total corrections applied for the blank enthalpy changes to be uncertain by $\approx \pm 0.2$ mJ and $\approx \pm 0.8$ mJ for reactions (2) and (3), respectively. Since the measured reaction enthalpy changes were, respectively, ≈−140 mJ and ≈−80 mJ for reactions (2) and (3), the uncertainties in the blank enthalpy changes lead to uncertainties of $\approx 0.002 \cdot \Delta_r H_m(\text{cal})$ and $\approx 0.010 \cdot \Delta_r H_m(\text{cal})$ in the final results for these two respective reactions. The quantity $\Delta_r H_m(\text{cal})$ is the calorimetrically determined molar enthalpy of reaction pertinent to the actual experimental conditions.

2.6. Measurement of pH

Measurement of pH was done with an Orion Model 811 pH meter and a Radiometer combination glass micro-electrode at the temperature at which experiments were performed. The pH meter was calibrated with Radiometer standard buffers that bracketed the pHs of the solutions used in this study. The pHs of the reaction mixtures were calculated by using interpolation with the measured electric potential differences and the pHs of the standard buffers.

3. Results and discussion

3.1. Thermodynamic formalism

The apparent equilibrium constants for reactions (2) and (3) are, respectively

$$K' = m(\text{AMP})/m(\text{cAMP}), \tag{5}$$

$$K' = m(\text{pyruvate}) \cdot m(\text{phosphate})/\{m(\text{PEP}) \cdot m^\circ\}. \quad (6)$$

The molalities m in the above equations are the total molalities of the various charged and uncharged species that are formed from the dissociation of the biochemical substances in solution. The quantity m° ($m^\circ = 1\ \text{mol} \cdot \text{kg}^{-1}$) has been used in equation (6) to make K' dimensionless. Following our usual practice, we have selected chemical reference reactions that involved the predominant species present under the experimental conditions used in our study. Thus, the selected reference reactions that correspond to the overall biochemical reactions (2) and (3) are, respectively

$$\text{cAMP}^-(\text{aq}) + \text{H}_2\text{O}(\text{l}) = \text{HAMP}^-(\text{aq}), \quad (7)$$

$$\text{PEP}^{3-}(\text{aq}) + \text{H}_2\text{O}(\text{l}) = \text{pyruvate}^-(\text{aq}) + \text{HPO}_4^{2-}(\text{aq}). \quad (8)$$

The equilibrium constants for these reference reactions are, respectively

$$K = m(\text{HAMP}^-)/m(\text{cAMP}^-), \quad (9)$$

$$K = m(\text{pyruvate}^-) \cdot m(\text{HPO}_4^{2-})/\{m(\text{PEP}^{3-}) \cdot m^\circ\}. \quad (10)$$

In this study, the standard state for the solute is the hypothetical ideal solution of unit molality ($m^\circ = 1\ \text{mol} \cdot \text{kg}^{-1}$) and the standard state of the solvent is the pure solvent; the standard pressure $p^\circ = 0.1$ MPa.

3.2. Results of experiments

The results of the extent of reaction measurements were that there were no measurable amounts of either cAMP or PEP in the reaction mixtures following the h.p.l.c. procedures described above (see Section 2.2). Based upon the lower limits of the amounts of these substances detectable with our h.p.l.c. instruments, it is possible to set lower limits for the values of the apparent equilibrium constants. Thus for reaction (2) $K' > 8 \cdot 10^3$ ($T = 298.15$ K, ionic strength $I_m = 0.32\ \text{mol} \cdot \text{kg}^{-1}$, pH = 7.53, pMg = 4.20) and for reaction (3) $K' > 450$ ($T = 298.15$ K, $I_m = 0.33\ \text{mol} \cdot \text{kg}^{-1}$, pH = 7.62). Such values are far outside the limits of our measurement capability. However, later in this paper, we will use thermochemical cycle calculations to establish the values of the equilibrium constants for these two reactions.

The results of the calorimetric measurements are (see tables 2 and 3): for reaction (2), the calorimetrically determined molar enthalpy of reaction $\Delta_r H_m(\text{cal}) = -(56.32 \pm 0.25)\ \text{kJ} \cdot \text{mol}^{-1}$ at pH = 7.53, $I_m = 0.32\ \text{mol} \cdot \text{kg}^{-1}$, and pMg = 4.20 and for reaction (3), $\Delta_r H_m(\text{cal}) = -(32.36 \pm 0.16)\ \text{kJ} \cdot \text{mol}^{-1}$ at pH = 7.62 and $I_m = 0.33\ \text{mol} \cdot \text{kg}^{-1}$. The uncertainties given here are equal to two estimated standard deviations of the mean. We judge that reasonable estimates of possible systematic error in the values of $\Delta_r H_m(\text{cal})$ for reaction (2) are: $0.003 \cdot \Delta_r H_m(\text{cal})$ due to impurities in the sample of cAMP; $0.002 \cdot \Delta_r H_m(\text{cal})$ due to possible calorimetric errors; $0.001 \cdot \Delta_r H_m(\text{cal})$ due to possible decomposition of cAMP prior to reaction; and $0.0003 \cdot \Delta_r H_m(\text{cal})$ due to a possible error in the extent of reaction. Similarly, we judge that reasonable estimates of possible systematic error in the values of

TABLE 2
Results of the calorimetric measurements for reaction (2) {cAMP(aq) + H_2O(l) = AMP(aq)} in phosphate buffer at $T = 298.15$ K, pH = 7.53, a calculated ionic strength $I_m = 0.32$ mol · kg^{-1}, and calculated pMg = 4.20

Experiment	$m(K_2HPO_4)$ / (mol · kg^{-1})	$m(H_3PO_4)$ / (mol · kg^{-1})	$10^3 \cdot m(MgCl_2)$ / (mol · kg^{-1})	$10^4 \cdot m$(Imidazole) / (mol · kg^{-1})	$10^3 \cdot m$(cAMP) / (mol · kg^{-1})	$\Delta_r H_m$(cal) / (kJ · mol^{-1})
1	0.1001	0.0138	1.07	5.23	2.945	−56.10
2	0.1001	0.0138	1.07	5.23	2.822	−56.87
3	0.1001	0.0138	1.07	5.23	2.987	−56.45
4	0.1001	0.0138	1.07	5.23	2.948	−56.25
5	0.1001	0.0138	1.07	5.23	2.804	−56.08
6	0.1001	0.0138	1.07	5.23	2.761	−56.12
	$\langle \Delta_r H_m(\text{cal}) \rangle = -(56.32 \pm 0.25)$ kJ · mol^{-1}					

Imidazole is $C_3H_4N_2$ and cAMP is the monosodium salt of adenosine 3′,5′-(cyclic)phosphate ($C_{10}H_{13}N_5NaO_7P$). The molalities m in columns 2 to 6 are those obtained after mixing of the enzyme and substrate solutions and prior to any reaction. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. The mass fraction w of the enzyme phosphodiesterase 3′,5′-cyclic nucleotide was ≈0.0014. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The uncertainty in the average value of $\Delta_r H_m$(cal) is equal to two estimated standard deviations of the mean.

TABLE 3
Results of the calorimetric measurements for reaction (3) {PEP(aq) + H_2O(l) = pyruvate(aq) + phosphate(aq)} in phosphate buffer at $T = 298.15$ K, pH = 7.62, and a calculated ionic strength $I_m = 0.33$ mol · kg^{-1}

Experiment	$m(K_2HPO_4)$ / (mol · kg^{-1})	$m(H_3PO_4)$ / (mol · kg^{-1})	$10^3 \cdot m$(PEP) / (mol · kg^{-1})	$\Delta_r H_m$(cal) / (kJ · mol^{-1})
1	0.1017	0.0141	2.752	−32.68
2	0.1017	0.0141	2.740	−32.23
3	0.1017	0.0141	2.784	−32.32
4	0.1017	0.0141	2.853	−32.37
5	0.1017	0.0141	2.824	−32.22
	$\langle \Delta_r H_m(\text{cal}) \rangle = -(32.36 \pm 0.16)$ kJ · mol^{-1}			

PEP is the monopotassium salt of phospho*enol*pyruvate ($C_3H_4KO_6P$). The molalities m in columns 2 to 4 are those obtained after mixing of the enzyme and substrate solutions and prior to any reaction. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The mass fraction w of the enzyme alkaline phosphatase was $\approx 4 \cdot 10^{-4}$. The uncertainty in the average value of $\Delta_r H_m$(cal) is equal to two estimated standard deviations of the mean.

$\Delta_r H_m$(cal) for reaction (3) are: $0.004 \cdot \Delta_r H_m$(cal) due to impurities in the sample of PEP; $0.005 \cdot \Delta_r H_m$(cal) due to possible calorimetric errors; $0.003 \cdot \Delta_r H_m$(cal) due to possible decomposition of PEP prior to reaction; and $0.0004 \cdot \Delta_r H_m$(cal) due to a possible error in the extent of reaction. These estimates of systematic error are combined in quadrature together with the statistical uncertainties in the measured values of $\Delta_r H_m$(cal) expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties [9]. These combined standard uncertainties were then multiplied by two to arrive at the final results (phosphate buffer and $T = 298.15$ K): $\Delta_r H_m(\text{cal}) = -(56.32 \pm 0.48)$ kJ · mol^{-1} for reaction (2) at pH = 7.53, $I_m = 0.32$ mol · kg^{-1}, and pMg = 4.20 and $\Delta_r H_m(\text{cal}) = -(32.36 \pm 0.48)$ kJ · mol^{-1} for reaction (3) at pH = 7.62 and $I_m = 0.33$ mol · kg^{-1}.

3.3. Equilibrium model

3.3.1. Selected values of pK and $\Delta_r H_m^\circ$ for H^+(aq) and metal ion dissociation reactions

The pKs and standard molar enthalpy $\Delta_r H_m^\circ$ changes for a variety of H^+(aq) and metal ion dissociation reactions for the reactants and for the buffers are needed to relate the experimental results for reactions (2) and (3) to thermodynamic quantities for the respective reference reactions (7) and (8). The reactions for which these quantities are needed are given in table 4 along with the selected values. Several of these reactions have been included because they are pertinent to the analysis of results from the literature. The basis of the selected values is now discussed. Where necessary, adjustment of pK and $\Delta_r H_m^\circ$ values to ionic strength $I_m = 0$ was done by using the extended Debye–Hückel equation [3] with the "ion-size" parameter set at 1.6 kg$^{1/2}$ · mol$^{-1/2}$. The reaction property values for reaction (11) and (52) are based on the values selected by Martell *et al.* [10] which we have adjusted to $I_m = 0$. The

TABLE 4
The pKs and standard molar enthalpy changes $\Delta_r H^\circ_m$ at $T = 298.15$ K and $I_m = 0$ for the aqueous H^+ and metal ion dissociation reactions of substances pertinent to this study and to the analysis of results from the literature

Reaction		pK	$\Delta_r H^\circ_m$ / (kJ · mol^{-1})
Pyruvic acid^0 = H^+ + pyruvate$^-$	(11)	2.48	12.1
H_2PEP^- = H^+ + $HPEP^{2-}$	(12)	3.83	−27[a]
$HPEP^{2-}$ = H^+ + PEP^{3-}	(13)	6.99	−16[a]
$MgPEP^-$ = Mg^{2+} + PEP^{3-}	(14)	3.45	−22[a]
H · cAMP = H^+ + $cAMP^-$	(15)	≈3.9	18[a]
Mg · cAMP$^+$ = Mg^{2+} + $cAMP^-$	(16)	1.15[a]	−21[a]
H_2AMP = H^+ + $HAMP^-$	(17)	3.99	18.1
$HAMP^-$ = H^+ + AMP^{2-}	(18)	6.73	−5.4
MgAMP = Mg^{2+} + AMP^{2-}	(19)	2.79	−11.3
H_2ADP^- = H^+ + $HADP^{2-}$	(20)	4.36	17.6
$HADP^{2-}$ = H^+ + ADP^{3-}	(21)	7.18	−5.6
$MgADP^-$ = Mg^{2+} + ADP^{3-}	(22)	4.65	−19.0
MgHADP = Mg^{2+} + $HADP^{2-}$	(23)	2.50	−12.5
H_2ATP^{2-} = H^+ + $HATP^{3-}$	(24)	4.68	15.0
$HATP^{3-}$ = H^+ + ATP^{4-}	(25)	7.60	−6.3
$MgATP^{2-}$ = Mg^{2+} + ATP^{4-}	(26)	6.18	−22.9
$MgHATP^-$ = Mg^{2+} + $HATP^{3-}$	(27)	3.63	−16.9
Mg_2ATP = Mg^{2+} + $MgATP^{2-}$	(28)	2.69	−10.8
$MgHPO_4$ = Mg^{2+} + HPO_4^{2-}	(29)	2.71	−12.2
$MgHP_2O_7^-$ = Mg^{2+} + $HP_2O_7^{3-}$	(30)	4.52	−11.8[a]
$MgP_2O_7^{2-}$ = Mg^{2+} + $P_2O_7^{4-}$	(31)	7.20	−13.3
$Mg_2P_2O_7$ = Mg^{2+} + $MgP_2O_7^{2-}$	(32)	3.91	−3.8[a]
$MgH_2P_2O_7$ = Mg^{2+} + $H_2P_2O_7^{2-}$	(33)	2.90	−11.1[a]
H_3PO_4 = H^+ + $H_2PO_4^-$	(34)	2.148	−8.0
$H_2PO_4^-$ = H^+ + HPO_4^{2-}	(35)	7.198	3.6
HPO_4^{2-} = H^+ + PO_4^{3-}	(36)	12.35	16.0
$H_3P_2O_7^-$ = H^+ + $H_2P_2O_7^{2-}$	(37)	2.26	−5.0
$H_2P_2O_7^{2-}$ = H^+ + $HP_2O_7^{3-}$	(38)	6.72	0.5
$HP_2O_7^{3-}$ = H^+ + $P_2O_7^{4-}$	(39)	9.46	1.4
H_2CO_3 = H^+ + HCO_3^-	(40)	6.351	9.15
HCO_3^- = H^+ + CO_3^{2-}	(41)	10.329	14.70

Table 4 (*continued*)

Reaction		pK	$\Delta_r H^\circ_m$ / (kJ · mol^{-1})
$H_2 \cdot GlyGly^+ = H^+ + H \cdot GlyGly^\pm$	(42)	3.140	0.11
$H \cdot GlyGly^\pm = H^+ + GlyGly^-$	(43)	8.265	43.4
$H \cdot Hepes^\pm = H^+ + Hepes^-$	(44)	7.564	20.4
$H \cdot Imidazole^+ = H^+ + Imidazole$	(45)	6.993	36.64
$H \cdot PIPES^\pm = H^+ + PIPES^-$	(46)	7.141	11.2
$H \cdot TAPS^\pm = H^+ + TAPS^-$	(47)	8.44	40.4
$H_2 \cdot Tricine^+ = H^+ + H \cdot Tricine^\pm$	(48)	2.023	5.85
$H \cdot Tricine^\pm = H^+ + Tricine^-$	(49)	8.135	31.37
$H \cdot TEA^+ = H^+ + TEA$	(50)	7.762	33.6
$H \cdot Tris^+ = H^+ + Tris$	(51)	8.072	47.45
$MnAMP = Mn^{2+} + AMP^{2-}$	(52)	3.25	−11.7
$MnHPO_4 = Mn^{2+} + HPO_4^{2-}$	(53)	3.49	−7.3[a]
$MgSO_4 = Mg^{2+} + SO_4^{2-}$	(54)	2.26	−20.1

See Section 3.3.1 for the basis of these values.

[a] Estimated.

[b] Where names and/or abbreviations have been used in this table, the chemical names and formulas of these substances are: GlyGly = Glycylglycine = $C_4H_7N_2O_3$; HEPES = *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid = $C_8H_{17}N_2O_4S$; Imidazole = $C_3H_4N_2$; PIPES = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) = $C_8H_{17}N_2O_6S_2$; pyruvic acid = $C_3H_4O_3$; TAPS = *N*-[tris(hydroxymethyl)methyl-3-amino]propanesulfonic acid = $C_7H_{16}NO_6S$; Tricine = *N*-tris(hydroxymethyl)methylglycine = $C_6H_{12}NO_5$; TEA = Triethanolamine = $C_6H_{15}NO_3$; and Tris = 2-amino-2-hydroxymethylpropane-1,3 diol = $C_4H_{11}NO_3$.

value of the pK for reaction (53) is also from Martell *et al.* [10]. The value of $\Delta_r H^\circ_m$ for this reaction was obtained by using the standard molar entropy change $\Delta_r S^\circ_m$ for reaction (29) together with the known pK value for reaction (53). The basis of the values used for reactions (12) and (13) has been given previously [5]. The value of the pK for reaction (14) is based on the result of Wold and Ballou [11] following adjustment to $I_m = 0$. We have estimated the value of $\Delta_r H^\circ_m$ for reaction (14) by taking $\Delta_r S^\circ_m$ for reaction (14) to be the average of the values of $\Delta_r S^\circ_m$ for reactions (22) and (27). The value of the pK for reaction (15) is based on the result of Tate [12] following adjustment to $I_m = 0$. The value of $\Delta_r H^\circ_m$ for reaction (15) was estimated by taking $\Delta_r S^\circ_m$ for reaction (15) to be the same value as $\Delta_r S^\circ_m$ for reaction (17). The value of the pK for reaction (16) is estimated from the result of Fan *et al.* [13] in which Mn^{2+}(aq) was used rather than Mg^{2+}(aq). The value of $\Delta_r H^\circ_m$ for this reaction was obtained by using the standard molar entropy change $\Delta_r S^\circ_m$ for reaction (19) together with the estimated pK value for reaction (16). The values of pK and $\Delta_r H^\circ_m$ for reaction (54) were calculated from the standard molar formation properties given by Wagman *et al.* [14]. The values of the thermodynamic quantities in the remainder

of the table are from Alberty and Goldberg [15] {reactions (17) to (29)}, Goldberg and Tewari [16] {reactions (30) to (33)}, and Goldberg *et al.* [17] {reactions (34) to (51)}.

3.3.2. Calculations

With the thermodynamic quantities given in table 4 it is now possible to use our previously described [3,4] equilibrium model to calculate values of $\Delta_r H^\circ_m$ ($T = 298.15$ K and $I_m = 0$) for the reference reactions (7) and (8) from the experimentally determined values of $\Delta_r H_m$(cal). The results of these calculations are $\Delta_r H^\circ_m = -47.8$ kJ·mol^{-1} and $\Delta_r H^\circ_m = -29.78$ kJ·mol^{-1} for reactions (7) and (8), respectively. The calculated values of $\Delta_r N(H^+)$, the changes in binding of H^+(aq) accompanying the biochemical reactions (2) and (3), are −0.947 and 0.0774, respectively. For reaction (2), the calculated value of $\Delta_r N(Mg^{2+})$, the change in binding of Mg^+(aq), is $5.6 \cdot 10^{-4}$. The ionic strengths of the reaction mixtures and the value pMg = 4.20 for reaction (2) were also calculated by using the equilibrium model.

The uncertainties in the calculated values of $\Delta_r H^\circ_m$ for the reference reactions have two components: the experimental uncertainties in the measured values of $\Delta_r H_m$(cal) (see Section 3.3) and possible errors in the quantities used in the equilibrium model. This latter component of uncertainty was examined by perturbing each of the pertinent quantities in the model by an assumed possible error in its value. The perturbations used for the pK values of the various species are: ±0.2 for H·cAMP(aq); ±0.2 for Mg·cAMP(aq); ±0.05 for H_2AMP(aq); ±0.05 for HAMP$^-$(aq); ±0.1 for MgAMP(aq); ±0.1 for H_2PEP^-(aq); ±0.1 for $HPEP^{2-}$(aq); ±0.03 for pyruvic acid (aq); ±0.003 for H_3PO_4(aq); ±0.003 for $H_2PO_4^-$(aq); ±0.03 for HPO_4^{2-}(aq); ±0.005 for $MgHPO_4$(aq); and ±0.003 for H·imidazole$^+$(aq). The perturbations used for the values of $\Delta_r H^\circ_m$/(kJ·mol^{-1}) are: ±6 for H·cAMP(aq); ±8 for Mg·cAMP(aq); ±2 for H_2AMP(aq); ±2 for HAMP$^-$(aq); ±4 for MgAMP(aq); ±7 for H_2PEP^-(aq); ±5 for $HPEP^{2-}$(aq); ±2 for pyruvic acid(aq); ±0.2 for H_3PO_4(aq); ±0.2 for $H_2PO_4^-$(aq); ±2 for HPO_4^{2-}(aq); ±0.4 for $MgHPO_4$(aq); and ±0.2 for H·imidazole$^+$(aq). The "ion-size" parameter used in the activity coefficient model was also perturbed by ±0.3 kg$^{1/2}$·mol$^{-1/2}$. The combined effects of these perturbations were ±1.9 kJ·mol^{-1} and ±0.3 kJ·mol^{-1} in the calculated values of $\Delta_r H^\circ_m$ for the reference reactions (7) and (8), respectively. In the calculation of the uncertainty in $\Delta_r H^\circ_m$ for reaction (7), the most significant factor was the uncertainty in the value of $\Delta_r H^\circ_m$ for the ionization of HAMP$^-$(aq) {reaction (18)}. In the calculation of the uncertainty in $\Delta_r H^\circ_m$ for reaction (8), the most significant factors were the uncertainties in the values of the pK and $\Delta_r H^\circ_m$ for the ionization of $HPEP^{2-}$(aq) {reaction (13)}. These estimated uncertainties were combined in quadrature with the final experimental uncertainties of ±0.48 kJ·mol^{-1} for both reactions (2) and (3) to obtain the final results: $\Delta_r H^\circ_m = -(47.8 \pm 2.0)$ kJ·mol^{-1} and $\Delta_r H^\circ_m = -(29.78 \pm 0.55)$ kJ·mol^{-1} for reactions (7) and (8), respectively, at $T = 298.15$ K and $I_m = 0$. The latter value will be rounded to $\Delta_r H^\circ_m = -(29.8 \pm 0.6)$ kJ·mol^{-1} in all future discussions.

3.4. Comparisons with previous results

There have been three previous calorimetric studies of reaction (2) [18–20] and two earlier calorimetric studies of reaction (3) [21,22]. The results of these studies are given in table 5. The early result of Greengard *et al.* [18] is clearly in error and was essentially acknowledged to be so by Gerlt *et al.* [20]. More importantly, there is a good agreement of our result with that obtained by Gerlt *et al.* [20]. The previous studies [21,22] of reaction (3) gave few details concerning the conditions of measurement. Indeed, George *et al.* [22] reported only the value $\Delta_r H^\circ_m = -25.1\ \mathrm{kJ \cdot mol^{-1}}$ for the reference reaction (8) and gave no other details. In any case, it is likely that there is agreement between our result and the very early result of Meyerhof and Schulz [21].

3.5. Standard molar formation properties

A thermochemical pathway to the standard molar enthalpies of formation $\Delta_f H^\circ_m$, standard molar Gibbs free energies of formation $\Delta_f G^\circ_m$, and standard partial molar entropies S°_m of the cAMP(aq) and PEP(aq) species is now described. The pathway uses measured thermodynamic quantities {K', $\Delta_r H_m$(cal), and the standard molar transformed enthalpy change $\Delta_r H'^\circ_m$} for reaction (1) and for the following biochemical reactions:

$$\mathrm{ATP(aq) + pyruvate(aq) = ADP(aq) + PEP(aq)}, \tag{55}$$

$$\mathrm{ATP(aq) + pyruvate(aq) + phosphate(aq) = AMP(aq) + PEP(aq) + pyrophosphate(aq)}. \tag{56}$$

The selected reference reactions corresponding to the above biochemical reactions are, respectively

$$\mathrm{ATP^{4-}(aq) = cAMP^{-}(aq) + HP_2O_7^{3-}(aq)}, \tag{57}$$

$$\mathrm{ATP^{4-}(aq) + pyruvate^{-}(aq) = ADP^{3-}(aq) + PEP^{3-}(aq) + H^{+}(aq)}, \tag{58}$$

$$\mathrm{ATP^{4-}(aq) + pyruvate^{-}(aq) + HPO_4^{2-}(aq) = AMP^{2-}(aq) + PEP^{3-}(aq) + HP_2O_7^{3-}(aq) + H^{+}(aq)}. \tag{59}$$

Using the equilibrium model [3,4] with the experimental results {K' and $\Delta_r H_m$(cal)} for reactions (1), (55), and (56) and with the pK and $\Delta_r H^\circ_m$ values given in table 4, we have calculated values of $\Delta_r H^\circ_m$, K, and $\Delta_r G^\circ_m$ for the reference reactions (57) to (59). The results of these calculations are given in table 6. Auxiliary property values ($\Delta_f H^\circ_m$, $\Delta_f G^\circ_m$, and S°_m) are also needed for several additional species in order to calculate the formation properties. The sources of these values are: Cox *et al.* [36] for H_2O(l), HPO_4^{2-}(aq), and Mg^{2+}(aq); Tewari and Goldberg [37] for pyruvate$^-$(aq); Goldberg and Tewari [16] for $HP_2O_7^{3-}$(aq), and Boerio-Goates *et al.* [38] for AMP^{2-}(aq), $HAMP^-$(aq), ADP^{3-}(aq), and ATP^{4-}(aq). The results of the calculations (see table 7) that follow pertain to $T = 298.15$ K and $I_m = 0$.

TABLE 5
Calorimetric results from the literature leading to values (column 8) of the standard molar enthalpy changes $\Delta_r H^\circ_m$ for the reference reactions (7) {$cAMP^-(aq) + H_2O(l) = HAMP^-(aq)$} and (8) {$PEP^{3-}(aq) + H_2O(l) = pyruvate^-(aq) + HPO_4^{2-}(aq)$} at the temperature $T = 298.15$ K and the ionic strength $I_m = 0$

Investigators	T/K	Buffer	pH	pMg	I_m / (mol · kg^{-1})	$\Delta_r H_m$(cal) / (kJ · mol^{-1})	$\Delta_r H^\circ_m$ / (kJ · mol^{-1})
Biochemical reaction (2): $cAMP(aq) + H_2O(l) = AMP(aq)$							
Reference reaction (7): $cAMP^-(aq) + H_2O(l) = HAMP^-(aq)$							
Greengard *et al.* (1969)[a] [18]	298.15	phosphate	7.3	3.89	0.10	−(63.0 ± 1.4)	−55.1
Cheung *et al.* (1974)[b] [19]	298.15	phosphate	7.8	[b]	0.15	−(61.1 ± 1.0)	−52.4
Gerlt *et al.* (1975)[a] [20]	298.15	phosphate	7.3	3.89	0.10	−(54.8 ± 0.6)	−46.9
This study	298.15	phosphate	7.53	4.20	0.32	−(56.3 ± 0.5)	−(47.8 ± 2.0)
Biochemical reaction (3): $PEP(aq) + H_2O(l) = pyruvate(aq) + phosphate(aq)$}							
Reference reaction (8): {$PEP^{3-}(aq) + H_2O(l) = pyruvate^-(aq) + HPO_4^{2-}(aq)$}							
Meyerhof and Schulz (1935)[c] [21]	293.15	phosphate	?	?	?	−35.4	≈−31
George *et al.* (1970)[d] [22]	298.15	?	?	?	?	?	−25.1
This study	298.15	phosphate	7.62	[e]	0.33	−(32.4 ± 0.5)	−(29.8 ± 0.6)

The calculated values of pMg and the ionic strength I_m are given in columns 5 and 6, respectively; the values of the calorimetrically determined molar enthalpy of reaction $\Delta_r H_m$(cal) corresponding to the overall biochemical reactions (2) and (3) are given in column 7. The basis of the uncertainties of our results is given in the text (see Section 3). The uncertainties given for the results of the remaining investigations reflect only the statistical uncertainties based on two estimated standard deviations of the mean.

[a] Both Greengard *et al.* [18] and Gerlt *et al.* [20] reported values of the standard molar transformed enthalpy change $\Delta_r H'^\circ_m$ for reaction (2). The values of $\Delta_r H_m$(cal) given above were obtained by using both their [18,20] reported values of $\Delta_r H'^\circ_m$ and their reported values of the buffer protonation corrections.

[b] Cheung *et al.* [19] in their table 5 give values for the "enthalpy of hydrolysis of cAMP." These values, however, were obtained "after correction for protonation of the phosphate buffer." The value $\Delta_r H_m(cal) = -61.1$ kJ · mol^{-1} was obtained from their [19] reported enthalpies of reaction and the amounts of cAMP(aq) that were hydrolyzed. They [19] also used $Mn^{2+}(aq)$ as a cofactor. The calculated value of pMn for their experiments is 5.7.

[c] In the calculation of $\Delta_r H^\circ_m$ from Meyerhof and Schulz's [21] reported value of $\Delta_r H_m$(cal), the following conditions were assumed: pH = 7.0; $m(MgCl_2) = 0.001$ mol · kg^{-1}; $I_m = 0.25$ mol · kg^{-1}. No correction was made to adjust the result to $T = 298.15$ K.

[d] George *et al.* [22] reported only the value of $\Delta_r H^\circ_m$ at $T = 298.15$ K and $I = 0$. No other details were given.

[e] Magnesium was not used in these experiments.

TABLE 6
Values of the standard molar enthalpy change $\Delta_r H^\circ_m$, equilibrium constant K, and standard molar Gibbs free energy change $\Delta_r G^\circ_m$ for the reference reactions (57) to (59) at the temperature $T = 298.15$ K and the ionic strength $I_m = 0$

Investigators	T/K	Buffer	pH	pMg	I_m / (mol · kg^{-1})	$\Delta_r H^\circ_m$ / (kJ · mol^{-1})	K	$\Delta_r G^\circ_m$ / (kJ · mol^{-1})
			Biochemical reaction (1): ATP(aq) = cAMP(aq) + pyrophosphate(aq)					
			Reference reaction (57): $ATP^{4-}(aq) = cAMP^{-}(aq) + HP_2O_7^{3-}(aq)$					
Hayaishi *et al.* (1971) [23][a]	298.15	Tris	7.3	2.80	0.11		0.47	1.87
Takai *et al.* (1971) [24][a,b]	306.15	Tris	7.3	2.91	0.058		1.1	−0.23
Kurashina *et al.* (1974) [25][a,c]	298.15	HEPES	6.2 to 7.7	3.64 to 4.44	0.057 to 0.098	29.0	0.81 ± 0.09	0.52 ± 0.25
			Biochemical reaction (55): ATP(aq) + pyruvate(aq) = ADP(aq) + PEP(aq)					
			Reference reaction (58): $ATP^{4-}(aq) + pyruvate^{-}(aq) = ADP^{3-}(aq) + PEP^{3-}(aq) + H^{+}(aq)$					
Meyerhof and Oesper (1949) [26][d,e]	303.15		≈7.6				$\approx 3 \cdot 10^{-10}$	≈54.7
Krimsky (1959) [27][e]	303.15	Tris	7.72 to 8.70	4.95	0.25		$(6.3 \pm 1.7) \cdot 10^{-12}$	63.9 ± 0.7
McQuate and Utter (1959) [28][e]	303.15	Tris	7.4 to 9.0	4.43	0.26		$(5.6 \pm 1.7) \cdot 10^{-12}$	64.2 ± 0.7
Rao *et al.* (1979) [29][e]	288.15	HEPES	8.0	4.38	0.40		$3.4 \cdot 10^{-12}$	65.4
Cheer *et al.* (1980) [30][f]	298.15	HEPES, TEA, Tris	8.00 to 8.54	5.12	0.093 to 0.24	6.6		
Redman-Furey (1982) [31][g]	298.15	HEPES, TAPS, glycylglycine, Tris	8.5	2.07	0.11 to 0.13	11.4		

	Biochemical reaction (56): ATP(aq) + pyruvate(aq) + phosphate(aq) = AMP(aq) + PEP(aq) + pyrophosphate(aq)						
	Reference reaction (59): $ATP^{4-}(aq) + pyruvate^{-}(aq) + HPO_4^{2-}(aq) = AMP^{2-}(aq) + PEP^{3-}(aq) + HP_2O_7^{3-}(aq) + H^+(aq)$						
Hatch and Slack (1968) [32][h]	303.15	Tris	8.3	2.37	0.042	$8.6 \cdot 10^{-12}$	63.3
Reeves *et al.* (1968) [33]	298.15	imidazole and Tris	6.51 to 8.39	1.9 to 6.1	0.081 to 0.16	$(4.7 \pm 3.0) \cdot 10^{-10}$	53.2 ± 1.6
Sugiyama (1973) [34]	298.15	Tris	7.24	4.99	0.077	$5.1 \cdot 10^{-10}$	53.1

The values of $\Delta_r H^\circ_m$, K, and $\Delta_r G^\circ_m$ (columns 7, 8, and 9, respectively) were calculated (see Section 3.5) from the reported values of the apparent equilibrium constants K', calorimetrically determined molar enthalpies of reaction $\Delta_r H_m$(cal), and, in one case [25], values of the standard molar transformed enthalpy change $\Delta_r H'^\circ_m$ for the (overall) biochemical reactions that correspond to the chemical reference reactions. The values of pMg and I_m were calculated by using the equilibrium model and the reported solution compositions. The uncertainties reflect only the statistical uncertainties based on two estimated standard deviations of the mean.

[a] The three equilibrium studies for reaction (1) cited here were carried out by Hayaishi *et al.* [23–25]. The final study [25], which was carried out in 1974, is assumed to be the most definitive.

[b] A value of $\Delta_r H^\circ_m = 29.0\ kJ \cdot mol^{-1}$ for reaction (57) at $T = 298.15$ K and $I = 0$ was used to adjust the result to $T = 298.15$ K.

[c] Kurashina *et al.* [25] measured K' as a function of temperature at two different pHs (7.0 and 7.30) and reported the results in terms of a small graph which does not allow an accurate recovery of the original values. However, they [25] did report the values of $\Delta_r H'^\circ_m$ that they calculated from these results. The value of $\Delta_r H^\circ_m$ given in column 7 for reaction (57) was calculated from their reported values of $\Delta_r H'^\circ_m$.

[d] Burton and Krebs [35], based on a personal communication from Meyerhof, give a pH of ≈7.6 for this study. The buffer, pMg, and I_m are not known.

[e] A value of $\Delta_r H^\circ_m = 9.3\ kJ \cdot mol^{-1}$ for reaction (58) at $T = 298.15$ K and $I = 0$ was used to adjust the result to $T = 298.15$ K.

[f] Cheer *et al.* [30] used Hepes, triethanolamine (TEA), and Tris buffers in their study. Use of their [30] reported values of $\Delta_r H_m$(cal) and our equilibrium model (see Section 3.3) leads to the following values for $\Delta_r H^\circ_m/(kJ \cdot mol^{-1})$ obtained with the various buffers: Hepes, 5.6; TEA, 6.1; and Tris, 8.2_3. These values are within ≈1 $kJ \cdot mol^{-1}$ of the values of $\Delta_r H^\circ_m$ calculated by Cheer *et al.* [30]. The average value $\Delta_r H^\circ_m = 6.6\ kJ \cdot mol^{-1}$ for the reference reaction (58) is given above.

[g] Use of Redman-Furey's [31] reported values of $\Delta_r H_m$(cal) and our equilibrium model leads to the following values for $\Delta_r H^\circ_m/(kJ \cdot mol^{-1})$ with the various buffers she used: glycylglycine, 15.6; TAPS, 11.7; Tricine, 11.9; and Tris, 10.7. We have discarded the result obtained with glycylglycine buffer and, in this table, give the average of the remaining values, $\Delta_r H^\circ_m = 11.4\ kJ \cdot mol^{-1}$ for the reference reaction (58) at $T = 298.15$ K and $I_m = 0$.

[h] A value of $\Delta_r H^\circ_m = 4.6\ kJ \cdot mol^{-1}$ for reaction (59) at $T = 298.15$ K and $I = 0$ was used to adjust the result to $T = 298.15$ K.

TABLE 7
Standard molar enthalpies of formation $\Delta_f H^\circ_m$, standard molar Gibbs free energies of formation $\Delta_f G^\circ_m$, and standard partial molar entropies S°_m for the adenosine 3′,5′-(cyclic)phosphate (cAMP) and phospho*enol*pyruvate (PEP) aqueous species at $T = 298.15$ K and $I_m = 0$

Species	Empirical formula	$\Delta_f H^\circ_m$	$\Delta_f G^\circ_m$	S°_m	Reference
		($kJ \cdot mol^{-1}$)	($kJ \cdot mol^{-1}$)	($J \cdot K^{-1} \cdot mol^{-1}$)	
H · cAMP	$C_{10}H_{12}N_5O_6P$	1314.3[a]	−816.0	304.5[a]	This study
$cAMP^-$	$C_{10}H_{11}N_5O_6P^-$	−1296.3	−793.7	290.2	This study
Mg · $cAMP^+$	$C_{10}H_{11}MgN_5O_6P^-$	−1724.3[a]	−1255.7[a]	245.6[a]	This study
H_2 PEP^-	$C_3H_4O_6P^-$	−1534.4[a]	−1333.1	325.6[a]	This study
$HPEP^{2-}$	$C_3H_3O_6P^{2-}$	−1561.4[a]	−1311.3	161.8[a]	This study
PEP^{3-}	$C_3H_2O_6P^{3-}$	−1577.4	−1271.4	−25.7	This study
$MgPEP^-$	$C_3H_2MgO_6P^-$	−2022.4[a]	−1746.5	−22.9[a]	This study
		Auxiliary property values			
H_2O(l)	H_2O	−285.83	−237.14	69.95	[36]
HPO_4^{2-}	HPO_4^{2-}	−1299.0	−1096.0	−33.5	[36]
$HP_2O_7^{3-}$	$HP_2O_7^{3-}$	−2294.87	−1973.86	−15.2	[16]
$pyruvate^-$	$C_3H_3O_3^-$	−594.0	−473.6	183	[37]
AMP^{2-}	$C_{10}H_{12}N_5O_7P^{2-}$	−1635.37	−1040.45	214.5	[38]
$HAMP^-$	$C_{10}H_{13}N_5O_7P^-$	−1629.97	−1078.86	361.4	[38]
ADP^{3-}	$C_{10}H_{12}N_5O_{10}P_2^{3-}$	−2626.54	−1906.13	207.4	[38]
ATP^{4-}	$C_{10}H_{12}N_5O_{13}P_3^{4-}$	−3619.21	−2768.10	182.8	[38]
Mg^{2+}	Mg^{2+}	−467.00	−455.4	−137.0	[36]

The auxiliary values used to calculate the formation properties of the cAMP and PEP species are also given. Abbreviations are: AMP, adenosine 5′-monophosphate; ADP, adenosine 5′-diphosphate; and ATP, adenosine 5′-triphosphate. The standard state is the hypothetical ideal solution of unit molality.

[a] This value is based, in part, on an estimate.

Our result, $\Delta_r H^\circ_m = -47.8$ $kJ \cdot mol^{-1}$ for reaction (7), is used in conjunction with the values of $\Delta_f H^\circ_m$ for H_2O(l) and for $HAMP^-$(aq) to calculate $\Delta_f H^\circ_m = -1296.34$ $kJ \cdot mol^{-1}$ for $cAMP^-$(aq). Use of this value together with the values of $\Delta_f H^\circ_m$ for ATP^{4-}(aq) and for $HP_2O_7^{3-}$(aq) leads to $\Delta_r H^\circ_m = 28.0$ $kJ \cdot mol^{-1}$ for reaction (57). This result is in excellent agreement with the value $\Delta_r H^\circ_m = 29.0$ $kJ \cdot mol^{-1}$ obtained from the study of Kurashina *et al.* [25] in which the temperature dependence of K' for reaction (1) was measured. Similarly, we use the value $\Delta_r G^\circ_m = 0.52$ $kJ \cdot mol^{-1}$ for reaction (57), which we calculated from the results of Kurashina *et al.* [25], together with the values of $\Delta_f G^\circ_m$ for ATP^{4-}(aq) and for $HP_2O_7^{3-}$(aq) to calculate $\Delta_f G^\circ_m = -793.72$ $kJ \cdot mol^{-1}$ for $cAMP^-$(aq). The standard partial molar entropy $S^\circ_m = 290.2$ $J \cdot K^{-1} \cdot mol^{-1}$ of $cAMP^-$(aq) is calculated by using the values of $\Delta_r H^\circ_m$ and $\Delta_r G^\circ_m$ for reaction (57) together with the values of S°_m for ATP^{4-}(aq) and for $HP_2O_7^{3-}$(aq). As a first approximation, one could estimate $S^\circ_m\{cAMP^-(aq)\} \approx (S^\circ_m\{HAMP^-(aq)\} - S^\circ_m\{H_2O(l)\} = 291$ $J \cdot K^{-1} \cdot mol^{-1}$. The excellent accord of the experimental result for S°_m with this estimate combined with the excellent agreement in the values of $\Delta_r H^\circ_m$ for reaction (57) lends some confidence to the values of the standard molar formation properties of $cAMP^-$(aq) given in table 7 and in the standard reaction quantities for reaction (57).

The value $\Delta_f H^\circ_m = -1577.37$ $kJ \cdot mol^{-1}$ for PEP^{3-}(aq) is calculated by using our result $\Delta_r H^\circ_m = -29.8$ $kJ \cdot mol^{-1}$ for reaction (8) together the values of $\Delta_f H^\circ_m$ for

TABLE 8
Values of standard molar enthalpy changes $\Delta_r H^\circ_m$, equilibrium constants K, and standard molar Gibbs free energy changes $\Delta_r G^\circ_m$ at $T = 298.15$ K and $I_m = 0$ for the several reference reactions considered herein

Reaction		$\Delta_r H^\circ_m$ / (kJ · mol^{-1})	K	$\Delta_r G^\circ_m$ / (kJ · mol^{-1})	$\Delta_r S^\circ_m$ / (J · K · mol^{-1})
$cAMP^-(aq) + H_2O(l) = HAMP^-(aq)$	(7)	−47.8	$2.6 \cdot 10^8$	−48.0	1.3
$PEP^{3-}(aq) + H_2O(l) = pyruvate^-(aq) + HPO_4^{2-}(aq)$	(8)	−29.8	$5.0 \cdot 10^{10}$	−61.1	105.3
$ATP^{4-}(aq) = cAMP^-(aq) + HP_2O_7^{3-}(aq)$,	(57)	28.0	0.81	0.52	92.2
$ATP^{4-}(aq) + pyruvate^-(aq) = ADP^{3-}(aq) + PEP^{3-}(aq) + H^+(aq)$,	(58)	9.3	$5.7 \cdot 10^{-12}$	64.2	−184.1
$ATP^{4-}(aq) + pyruvate^-(aq) + HPO_4^{2-}(aq) = AMP^{2-}(aq) + PEP^{3-}(aq) + HP_2O_7^{3-}(aq) + H^+(aq)$.	(58)	4.6	$7.7 \cdot 10^{-10}$	52.0	−158.7

The standard state is the hypothetical ideal solution of unit molality.

pyruvate$^-$(aq), HPO_4^{2-}(aq), and H_2O(l) given in table 7. Use of this value of $\Delta_f H_m^\circ$ for PEP^{3-}(aq) together with the values of $\Delta_f H_m^\circ$ for ATP^{4-}(aq), ADP^{3-}(aq), and pyruvate$^-$(aq) from table 7 leads to $\Delta_r H_m^\circ = 9.3\ \text{kJ}\cdot\text{mol}^{-1}$ for reaction (58). This value is in satisfactory agreement with the values (see table 6) of $\Delta_r H_m^\circ$ that range from $6.6\ \text{kJ}\cdot\text{mol}^{-1}$ to $11.4\ \text{kJ}\cdot\text{mol}^{-1}$ that we calculated from the calorimetric results of Cheer *et al.* [30] and Redman-Furey [31]. The two routes to the formation properties of PEP^{3-}(aq) use values of $\Delta_r G_m^\circ$ (see table 6) for reactions (58) and (59) together with values of $\Delta_f G_m^\circ$ for ATP^{4-}(aq), ADP^{3-}(aq), pyruvate$^-$(aq), AMP^{2-}(aq), $HP_2O_7^{3-}$(aq), and HPO_4^{2-}(aq). For reaction (58), we adopt the value $\Delta_r G_m^\circ = 64.2\ \text{kJ}\cdot\text{mol}^{-1}$ based primarily on the study of McQuate and Utter [28]; this result leads to $\Delta_f G_m^\circ = -1271.4\ \text{kJ}\cdot\text{mol}^{-1}$ for PEP^{3-}(aq). For reaction (59), we adopt the value $\Delta_r G_m^\circ = 53.2\ \text{kJ}\cdot\text{mol}^{-1}$ based on the studies of Reeves *et al.* [33] and of Sugiyama [34]; this result leads to $\Delta_f G_m^\circ = -1270.2\ \text{kJ}\cdot\text{mol}^{-1}$ for PEP^{3-}(aq). Considering the uncertainties of the several values that entered into the calculations, this is judged to be a satisfactory agreement. Since reaction (58) appears to have been studied more thoroughly than reaction (59) and also because it relies on fewer auxiliary values of $\Delta_f G_m^\circ$, the route that uses reaction (58) is preferred and we adopt the value $\Delta_f G_m^\circ = -1271.4\ \text{kJ}\cdot\text{mol}^{-1}$ for PEP^{3-}(aq). The remaining property values for the cAMP and PEP species (see table 7) are calculated from the values just obtained together with the auxiliary values given in table 7 and the appropriate pK and $\Delta_r H_m^\circ$ values from table 4. The property values given in table 7 are now used to calculate a set of "best" values of $\Delta_r H_m^\circ$, K, $\Delta_r G_m^\circ$, and $\Delta_r S_m^\circ$ for the five reference reactions considered herein. These values are given in table 8.

3.6. Apparent equilibrium constants under approximately physiological conditions

It is important to appreciate that the results given in tables 4, 7, and 8 provide the essential property values needed to calculate K' and the position of equilibrium of the

TABLE 9
Values of the apparent equilibrium constants K' and standard molar transformed Gibbs free energy changes $\Delta_r G_m'^\circ$ at $T = 311.15$ K, pH = 7.0, pMg = 3.0, and $I_m = 0$ and $I_m = 0.25\ \text{mol}\cdot\text{kg}^{-1}$ for the several overall biochemical reactions considered herein

Reaction		I_m ($\text{mol}\cdot\text{kg}^{-1}$)	K'	$\Delta_r G_m'^\circ$ ($\text{kJ}\cdot\text{mol}^{-1}$)
ATP(aq) = cAMP(aq) + pyrophosphate(aq)	(1)	0	0.26	3.45
		0.25	0.17	4.55
cAMP(aq) + H_2O(l) = AMP(aq)	(2)	0	$5.2\cdot10^{7}$	−46.0
		0.25	$8.6\cdot10^{7}$	−47.3
PEP(aq) + H_2O(l) = pyruvate(aq) + phosphate(aq)	(3)	0	$1.5\cdot10^{10}$	−60.6
		0.25	$8.9\cdot10^{9}$	−59.3
ATP(aq) + pyruvate(aq) = ADP(aq) + PEP(aq)	(55)	0	$7.7\cdot10^{-6}$	30.5
		0.25	$1.6\cdot10^{-5}$	28.5
ATP(aq) + pyruvate(aq) + phosphate(aq) = AMP(aq) + PEP(aq) + pyrophosphate(aq)	(56)	0	$4.7\cdot10^{-4}$	19.8
		0.25	$1.2\cdot10^{-3}$	17.4

several biochemical reactions considered herein as a function of temperature, pH, pMg, and ionic strength. These calculations can be performed either by using a chemical equilibrium model [3,4] or by using the standard molar formation properties [15]. Of particular interest are the values of the apparent equilibrium constants under approximately physiological conditions which are taken to be [39] $T = 311.15$ K, pH = 7.0, pMg = 3.0, and $I_m = 0.25$ mol · kg^{-1}. By using the equilibrium model with the property values given in tables 4 and 8, we have calculated the values of K' and of the standard molar transformed Gibbs free energy change $\Delta_r G'^{\circ}_m$ under these conditions for the five overall biochemical reactions considered herein. These values are given in table 9.

Acknowledgements

We thank Dr. Prasad Reddy (National Institute of Standards and Technology) for helpful discussions regarding the biochemistry of the reaction that forms adenosine 3′,5′-(cyclic)phosphate from ATP.

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JCT 03-086