Biophysical Chemistry 12 (1980) 73–82

A MICROCALORIMETRIC STUDY OF THE REACTION CATALYSED BY PYRUVATE KINASE

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Received 9 November 1979
Revised manuscript received 6 February 1980

The enthalpy change for phosphorylation of ADP^{3-} by PEP^{3-} catalysed by pyruvate kinase has been determined at 25°C using flow microcalorimetry. Measurements were made at pH 8 in three buffer systems TRIS, TEA and HEPES and also at pH 8.5 in TRIS buffer. The values of ΔH obtained, -8.7_5 kJ mol^{-1} in TRIS, -7.3_9 kJ mol^{-1} in TEA and -6.1_9 kJ mol^{-1} in HEPES surprisingly display a dependence on the buffer system used. The enthalpy change was combined with free energy data to calculate the entropy change for the catalysed reaction.

1. Introduction

Though free energy changes (ΔG) for enzyme-catalysed reactions in metabolic pathway are available for many such reactions [1,2], data on the corresponding enthalpy (ΔH) and entropy (ΔS) changes are scarce. These data are useful in that they enable a more complete interpretation of the thermodynamics of a reaction. In this paper we report a calorimetric determination of the enthalpy change for the reaction catalysed by pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40). Pyruvate kinase, which is a key enzyme in the glycolytic pathway, catalyses the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine-5′-diphosphate (ADP) to form the products pyruvate (pyr) and adenosine-5′-triphosphate (ATP)

$$H^+ + ADP^{3-} + PEP^{3-} \rightarrow pyr^- + ATP^{4-}. \qquad (1)$$

The enzyme is also unique in that it has an absolute requirement for both a monovalent cation (K^+ in *vivo*) and a divalent ion (Mg^{2+} in *vivo*) for its catalytic activity [3].

To define the ionic states of both reactants and products the reaction was studied in the presence of a buffer. It was thought advisable to use more than one buffer system to ensure that there were no specific buffer effects. Measurements were made in three buffer systems, TRIS(tris(hydroxymethyl)aminomethane)/TRIS.HCl, TEA(triethanolamine)/TEA.HCl, and HEPES(N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid)/KOH.

2. Experimental

2.1. Materials

Samples of rabbit muscle pyruvate kinase (lyophilised salt-free material), obtained from either Sigma Chemical Co., or Worthington Biochemical Corporation, were used without further purification. The specific activity of each sample was determined using the method of Bucher and Pfleiderer [4]. The activities of the samples were somewhat variable, but were generally in the range 50–120 μmoles of product/min/mg enzyme at 20°C.

TEA.HCl, HEPES, the barium salt of ADP and the monopotassium salt of PEP were purchased from Sigma Chemical Co. and used without further purification.

Aristar grade TRIS, Analar grade KCl, and Reagent grade $MgCl_2 6H_2O$ were obtained from BDH Chemicals Ltd. The magnesium chloride was recrystallised three times from deionized water. The concentration of

magnesium ions in the stock solution prepared using the recrystallised material was determined by complexometric titration with standard ethylenediaminetetraacetic acid [5].

2.2. Preparation of solutions

All solutions were made up using doubly distilled water which was further degassed by boiling. Stock 0.05 mol dm^{-3} buffer solutions containing 0.1 mol dm^{-3} KCl and ca 2×10^{-3} mol dm^{-3} $MgCl_2$ were prepared. The pH of each buffer solution was determined using a Radiometer pHM64 digital pH meter.

ADP solutions were prepared by suspending samples of the insoluble barium salt in a volume of buffer solution, and stirring this with the potassium form of a cation exchange resin (Dowex-50, Sigma Chemical Co.). After the resin was removed by filtration the concentration of ADP was determined enzymatically using the pyruvate kinase/lactate dehydrogenase coupled assay [6]. The solutions were also checked for inorganic phosphate [7], but only small amounts (<0.5%) were detected. As an additional check on the purity of the ADP solutions, a sample was subjected to ion-exchange chromatography [8]. Calorimetric runs using ADP so purified gave results consistent with those using untreated samples (see table 1). It was therefore unnecessary to further purify each ADP solution.

Solutions of the enzyme and PEP were prepared by the addition of weighed amounts of each material to a known volume of buffer solution. In addition, for some experiments, the enzyme was pre-reacted by the addition of a small, known quantity of the ADP solution to the enzyme/PEP solution. This was to ensure that there was no detectable heat effect from enzyme-substrate binding and dissociation.

2.3. Calorimeter

Heat measurements were made with a LKB Model 10700-1 flow microcalorimeter. The design is essentially that described by Monk and Wadso [9]. A Keithley model 150B microvoltammeter and a Servogor Model RE512 chart recorder were used in conjunction with the microcalorimeter. LKB peristaltic pumps were used to pump the reactants through the flow cells. The flow rates (ca 4×10^{-3} cm^3 s^{-1}) were constant to 0.1% over many hours, and were determined at regular intervals by calibration using distilled water. The accuracy of the calorimeter was checked by determining the enthalpy of protonation of TRIS. The mean enthalpy change over a range of heat fluxes (0.15–0.9 mW) was -47.2 ± 0.3 kJ mol^{-1}, which is in satisfactory agreement with accepted literature data (-47.4_4 kJ mol^{-1}) [10].

2.4. Heat measurements

The enthalpy change for reaction (1), was calculated from the measured heats of mixing of the buffered solutions of ADP, with the buffered solutions of PEP and enzyme. The heats of mixing so measured are made up of the sum of the various heats of reaction which are associated with the chemical changes that occur, and also the heats of dilution of ADP, PEP and the enzyme. These dilution effects were corrected for as follows.

In separate experiments the PEP solutions used were diluted with the buffer solution. The observed heat fluxes were found to be negligible ($< 3 \times 10^{-4}$ mW). Prior to the mixing of the PEP and ADP solutions, the latter was mixed with the buffer solution until a steady heat flux, $\dot{Q}_1$ was obtained. The pure buffer solution was then replaced by the solution containing PEP and enzyme, and the mixing experiment continued until again a steady heat flux, $\dot{Q}_2$ was obtained. The difference between the two fluxes, $(\dot{Q}_2 - \dot{Q}_1)$ was ascribed to the chemical changes occurring and is the quantity used to calculate the enthalpy of reaction (1). For given concentrations of reactants, the observed heat flux did not change when the enzyme concentration was doubled, indicating that the reaction was catalysed to its completion well within the time the reactants were in the reaction cell of the calorimeter. This was also confirmed by spectroscopy using the pyruvate kinase/lactate dehydrogenase coupled assay on solutions whose reagent concentrations were similar to those in the calorimetric experiments.

The change of pH on mixing the solutions in the calorimetric experiments was always less than 0.02 units.

3. Results and discussion

3.1. Extent of reaction

The apparent equilibrium constant, K_{app}, for reaction (1) is given by

$$K_{app} = \frac{(ATP)(pyr)}{(ADP)(PEP)}, \quad (2)$$

where the parentheses represent the total concentrations in moles per litre of the various reactants and products. Values of K_{app} have been determined [11] under conditions closely similar to those used in this work; at pH 8.0 and 30°C K_{app} is 2150. Using this value of K_{app} and the pK_a (at 25°C) for each buffer [12] an apparent equilibrium constant K'_{app} can be calculated for the reaction

$$BH^+ + ADP + PEP \rightarrow ATP + pyr + B \quad (3)$$

$$K'_{app} = \frac{(ATP)(pyr)[B]}{(ADP)(PEP)[BH^+]} \quad (4)$$

where [B] and $[BH^+]$ represent the concentrations of the basic and acidic forms of the buffer respectively. The values of K'_{app} at pH 8 are 1707, 3720 and 6059 for the buffers TRIS, TEA and HEPES respectively. Hence, for the concentrations of reactants used in this study, reaction (3) goes to completion to greater than 99.9% in each buffer system. This has been assumed in the analysis of the calorimetric results.

3.2. Experimental data

The results of the mixing experiments are given in table A1 (See Appendix.) The tabulated concentrations are those for the mixed solution in the flow cell. In all experiments ADP was the limiting reagent. The column headed total ATP arises because of the pre-reaction in some experiments. The experimental data are given in full in case further information helpful to their interpretation becomes available at a later stage.

3.3. Analysis of results

The enthalpy change required, ΔH, is that for reaction (1). However, the observed heat flux, $\dot{Q}_{obs}$, results from the reaction

$$BH^+ + \sum PEP + \sum ADP \rightarrow \sum ATP + pyr^- + B, \quad (5)$$

where Σ indicates that there are different ionic and metal ion bound states for the species (e.g. under the experimental conditions used ADP will exist in the forms ADP^{3-}, $HADP^{2-}$, $KADP^{2-}$ and $MgADP^-$). The observed heat flux can be expressed

$$\dot{Q}_{obs} = n_{ADP} \cdot \Delta H + n_{BH^+} \cdot \Delta H_{buf} + \left[\sum_M n_{MATP} \cdot \Delta H_{MATP} - \sum_M n_{MADP} \cdot \Delta H_{MADP} - \sum_M n_{MPEP} \cdot \Delta H_{MPEP}\right] \quad (6)$$

where n_{ADP} is total number of moles of ADP reacting per second, n_{BH^+} is the number of moles of BH^+ reacting per second and n_{MADP} etc. refer to the number of moles of complexed or protonated species formed (or dissociated) per second. The enthalpy change ΔH_{buf} refers to the buffer ionisation

$$BH^+ \rightarrow B + H^+, \quad (7)$$

while ΔH_{MADP} etc. refers to the enthalpy change for the process

$$M^{n+} + ADP^{3-} \rightarrow MADP^{(3-n)-}, \quad (8)$$

where M^{n+} represents H^+, Mg^{2+} or K^+.

To correct for the terms within the square brackets in equation (6), the concentrations of the various complexed and protonated species before and after reaction must be calculated. These concentrations were determined by solving the following mass balance equations

$$T_{ATP} = [ATP^{4-}]\left(1 + \sum_M K_{MATP} \cdot [M]\right) \quad (9)$$

$$T_{ADP} = [ADP^{3-}]\left(1 + \sum_M K_{MADP} \cdot [M]\right) \quad (10)$$

$$T_{PEP} = [PEP^{3-}]\left(1 + \sum_M K_{MPEP} \cdot [M]\right) \quad (11)$$

$$T_{Mg} = [Mg^{2+}]\left(1 + \sum_L K_{MgL} \cdot [L]\right) \quad (12)$$

$$T_K = [K^+]\left(1 + \sum_L K_{KL} \cdot [L]\right) \quad (13)$$

where T_X is the total concentration of species X, L

represents ADP^{3-}, ATP^{4-} or PEP^{3-} and K_{MATP} etc. are the equilibrium constants for the formation of the complex or protonated species. The equilibrium constants used are shown in table 1. The equations were solved by an iterative method using a Burroughs B6700 computer. Using initial approximations for the ionic strength and the concentrations of uncomplexed Mg^{2+} and K^+ ions, and using the known hydrogen ion concentration, equations (9)–(11) were solved to give estimates for the concentrations of ATP^{4-}, ADP^{3-} and PEP^{3-}. These values were substituted into equations (12) and (13) to obtain improved values for the concentration of Mg^{2+} and K^+ ions. An improved value of the ionic strength was calculated and the process repeated until convergence was obtained. This usually took 7–9 cycles. The results of these calculations are shown in tables A2 and A3. For each experiment the first row of data refers to the concentrations of species before reaction and the second row refers to the concentrations of the species after the reaction.

The change in concentration for each species was then used to calculate the associated heat fluxes. The enthalpy data used are shown in table 1. For the protonation and complex formation reactions of ADP^{3-} and ATP^{4-} the standard enthalpy changes, ΔH^0, were corrected for ionic strength effects using [17]

$$\Delta H = \Delta H^0 - 2.303\,RT^2\left(\sum_i z_i^2(\text{prod}) - \sum_i z_i^2(\text{react})\right) \times \frac{\partial}{\partial t}\left(A\left(\frac{I^{1/2}}{1+I^{1/2}} - 0.3\,I\right)\right), \quad (14)$$

where z_i(prod) and z_i(react) are the ionic charges of the products and reactants respectively, A is the Debye-Huckel parameter and I is the ionic strength. The magnitude of each heat flux associated with changes in complex formation and protonation is shown in table A4. The contributions from $MgADP^-$ dissociation and $MgATP^{2-}$ formation are of similar magnitude to $\dot{Q}_{obs}$. Contributions to $\dot{Q}_{obs}$ from changes in potassium complex formation and protonation were negligible. In column 9, the quantity $\dot{Q}_{corr}$, which is the difference between $\dot{Q}_{obs}$ and the sum of the effects of complex formation, is a measure of the heat effects of reaction (1) and buffer dissociation. In column 10 the term ΔH_{corr} is just $\dot{Q}_{corr}$ divided by

Table 1
Equilibrium constant and enthalpy data used in the analysis of the calorimetric heat data. The temperature is 25.0°C

Reaction	Constant	log K [a]	ΔH^0/kJ mol^{-1}	Ref.
$H^+ + ADP^{3-} \rightleftharpoons HADP^{2-}$	K_{HADP}	$7.20 - 2.54\,I^{1/2} + 3.84\,I$	5.73	[13]
$H^+ + ATP^{4-} \rightleftharpoons HATP^{3-}$	K_{HATP}	$7.68 - 3.56\,I^{1/2} + 4.90\,I$	7.03	[13]
$H^+ + PEP^{3-} \rightleftharpoons HPEP^{2-}$	K_{HPEP}	6.35		[14]
$Mg^{2+} + ADP^{3-} \rightleftharpoons MgADP^-$	K_{MgADP}	$4.27 - 4.06\,I^{1/2} + 6.36\,I - \frac{2.04\,I^{1/2}}{1+6.02\,I^{1/2}}$	17.99	[13]
$Mg^{2+} + ATP^{4-} \rightleftharpoons MgATP^{2-}$	K_{MgATP}	$5.83 - 6.10\,I^{1/2} + 8.74\,I - \frac{2.04\,I^{1/2}}{1+6.02\,I^{1/2}}$	21.34	[13]
$Mg^{2+} + PEP^{3-} \rightleftharpoons MgPEP^-$	K_{MgPEP}	2.26 [b]	10.8 ± 0.2 [c]	[14]
$K^+ + ADP^{3-} \rightleftharpoons KADP^{2-}$	K_{KADP}	$2.18 - 6\left(0.509\left(\frac{I^{1/2}}{1+I^{1/2}} - 0.3\,I\right)\right)$	– [d]	[15]
$K^+ + ATP^{4-} \rightleftharpoons KATP^{3-}$	K_{KATP}	$2.36 - 8\left(0.509\left(\frac{I^{1/2}}{1+I^{1/2}} - 0.3\,I\right)\right)$	−0.5 ± 0.4 [c]	[16]
$K^+ + PEP^{3-} \rightleftharpoons KPEP^{2-}$	K_{KPEP}	1.08 [b]	0.1 ± 0.4 [c]	[14]

[a] I denotes the solution ionic strength.
[b] Determined at an ionic strength of 0.1 mol dm^{-3}.
[c] G.R. Hedwig unpublished results, I = 0.12 mol dm^{-3}.
[d] From the results for ATP^{4-} and PEP^{3-} this value was taken as zero.

n_{ADP}. From equation (6) it follows that

$$\Delta H_{corr} = \Delta H + (n_{BH^+}/n_{ADP}) \cdot \Delta H_{buf}. \quad (15)$$

For each buffer system ΔH_{corr} is constant, within the experimental uncertainty, as the total concentration of ADP varies. This is confirmation that reaction (1) proceeds essentially to completion. The results for the TRIS buffer system indicate that ΔH_{corr} is indeed pH independent as expected. The mean and standard deviation of ΔH_{corr} for each buffer system are included in table A4. The standard deviation from the mean of ca 2% is consistent with the experimental uncertainty in the determination of ΔH_{corr}

Due to the competition for protons amongst the various proton acceptor species the ratio r_{H^+} defined by the equation

$$r_{H^+} = n_{BH^+}/n_{ADP} \quad (16)$$

need not be exactly unity. This ratio was calculated by considering the mass balance for total ionisable acid before and after the enzyme catalysed reaction. At pH 8 such an analysis gives a value $r_{H^+} = 0.99 \pm 0.01$ and at pH 8.54, $r_{H^+} = 1.00 \pm 0.01$. The errors were assessed from the effect of the uncertainties in protonation constants, pH etc. on the value of r_{H^+}.

Using these values of r_{H^+} and the literature data for ΔH_{buf}, ΔH was calculated using equation (15). The results are shown in table 2. The variation in ΔH as the buffer changes is surprising. This variation is examined in more detail in fig. 1 where ΔH_{corr} is plotted against ΔH_{buf}. The dotted line, which corresponds to a slope of $r_{H^+} = 1.00$ is just outisde the experimental uncertainty of the measurements. The data can be fitted exactly if $r_{H^+} = 0.91$. This low value suggests that perhaps there is some other step occurring which involves protons.

Pyruvate kinase does have hydrolase activity and will catalyse the hydrolysis of PEP [20], but this activity is lower than that of the kinase by a factor of a thousand. Furthermore, the negligible heat of dilution of the PEP/enzyme solution suggests that the low r_{H^+} value does not arise from hydrolase activity. Unfortunately, our results are not of sufficient precision to assert that the value of $r_{H^+} = 0.91$ is correct as they could (just) be accomodated with a value of 0.96. Nonetheless we have used the value of 0.91 to calculate the ΔH value for reaction (1) of -4.4 ± 0.2 kJ mol^{-1}.

Table 2
Enthalpy data for the reaction catalysed by pyruvate kinase

Buffer	pH	ΔH_{corr} (kJ mol^{-1})	r_{H^+}	ΔH_{buf} (kJ mol^{-1})	ΔH (kJ mol^{-1})
HEPES	8.02	14.5_0	0.99	−20.9 [18]	-6.1_9
TEA	8.06	26.3_7	0.99	−34.1 [19]	-7.3_9
TRIS	8.00	38.1_8	0.99	−47.4 [10]	-8.7_5
TRIS	8.54	38.5_8	1.00	−47.4 [10]	-8.8_2

Thermodynamic data for reaction (1) and also for the hexokinase-catalysed reaction are shown in table 3. The large negative free energy change for the pyruvate-

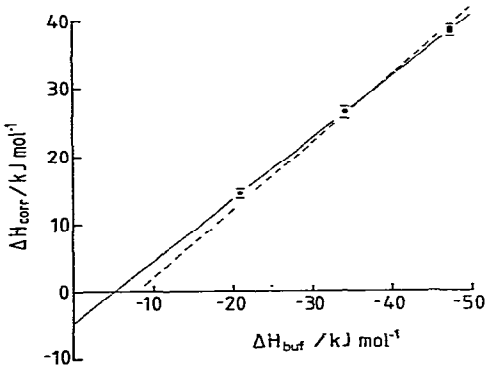

Fig. 1. ΔH_{corr} as a function of ΔH_{buf}. The error bars indicate the positions $\Delta H_{corr} + 2\sigma$ and $\Delta H_{corr} - 2\sigma$ where σ is the standard deviation of ΔH_{corr}.

Table 3
Thermodynamics of the pyruvate kinase-catalysed and hexokinase-catalysed reactions at 25°C [a]

Reaction	ΔG^0/kJ mol^{-1}	ΔH^0/kJ mol^{-1}	ΔS^0/J K^{-1} mol^{-1}
$H^+ + ADP^{3-} + PEP^{3-} \rightleftharpoons ATP^{4-} + pyr^-$	−65.6 [b]	−4.4 [c]	+205
$H^+ + ADP^{3-} + Gl\text{-}6\text{-}P^{2-} \rightleftharpoons ATP^{4-} + Gl$	−16.3 [22]	+23.8 [21]	+135

[a] The standard state is the hypothetical ideal solution of unit concentration.
[b] Calculated from the equilibrium data in ref. [11] and corrected to 25°C.
[c] This work, the value of the intercept in fig. 1.

catalysed reaction results almost entirely from the large positive entropy term. This clearly indicates the importance of ion-solvent interactions for highly charged anions. This has been noted previously for hydrolysis reactions of some phosphate esters including PEP and the adenine nucleotides [22].

In the hexokinase-catalysed reaction, where three species are the same as those in the pyruvate kinase reaction, although the entropy term is still large and positive the enthalpy term also makes a significant contribution to the overall free energy change.

It is clear that the difference in partial molar entropy for the glucose species, $\bar{S}^0_{Gl} - \bar{S}^0_{Gl\text{-}6\text{-}P^{2-}}$ is smaller than the corresponding difference for the pyruvate species, $\bar{S}^0_{pyr^-} - \bar{S}^0_{PEP^{3-}}$. Presumably this results from the "structure-making" effects of the high negative charge on the PEP^{3-} anion.

Acknowledgements

We thank Dr. G.R. Bruce for assistance with preliminary experiments and Professor G.N. Malcolm for helpful discussions. We acknowledge the support from the New Zealand Universities Grants Committee for the purchase of the microcalorimeter.

Appendix

Table A1
Experimental data for the pyruvate kinase catalysed reaction at 25.0 ± 0.1°C

Buffer	Expt No	pH	Total ADP (mM) [a]	Total PEP (mM)	Total ATP (mM)	Total Mg (mM)	Total K (M)	enzyme (mg cm^{-3})	$\dot{Q}_{obs}$ (mW)	Total flow rate (cm^3 min^{-1})
HEPES	1	8.02	0.7509	0.982	0.0145	1.930	0.1032	0.03	0.0920	0.4553
HEPES	2	8.02	0.7509	0.982	0.0145	1.930	0.1032	0.03	0.0933	0.4553
HEPES	3	8.02	0.7509	0.982	0.0145	1.930	0.1032	0.03	0.0932	0.4553
HEPES	4	8.02	0.6644	0.982	0.0058	1.930	0.1030	0.03	0.0833	0.4553
HEPES	5	8.02	0.6644	0.982	0.0058	1.930	0.1030	0.03	0.0825	0.4553
HEPES	6	8.02	0.5315	0.982	0.0058	1.930	0.1026	0.03	0.0648	0.4553
HEPES	7	8.02	0.5315	0.982	0.0058	1.930	0.1026	0.03	0.0667	0.4553
TEA	1	8.06	0.6909	0.988	–	1.929	0.1030	0.03	0.1485	0.4561
TEA	2	8.06	0.6146	0.988	–	1.929	0.1028	0.05	0.1338	0.4561
TEA	3	8.06	0.6146	0.988	–	1.929	0.1028	0.05	0.1333	0.4461
TEA	4	8.06	0.3075	0.988	–	1.929	0.1019	0.05	0.0675	0.4561
TEA	5	8.06	0.3490	0.988	–	1.929	0.1020	0.05	0.0745	0.4561
TEA	6	8.06	0.3490	0.988	–	1.929	0.1020	0.05	0.0755	0.4561
TEA	7	8.06	0.3490	0.988	–	1.929	0.1020	0.08	0.0755	0.4561
TRIS	1	8.00	0.7483	1.002	–	1.934	0.1033	0.05	0.2410	0.5012
TRIS	2	8.00	0.3821	1.002	0.0078	1.934	0.1022	0.03	0.1195	0.5012
TRIS	3	8.00	0.4739	1.002	0.0096	1.934	0.1024	0.03	0.1525	0.5012
TRIS	4	8.00	0.7134	1.002	0.0145	1.934	0.1031	0.03	0.2250	0.5012
TRIS	5	8.00	0.2671	0.984	0.0052	1.934	0.1018	0.03	0.0797	0.4400
TRIS	6	8.00	0.7915	0.984	0.0155	1.934	0.1034	0.03	0.2395	0.4400
TRIS	7	8.54	0.3400	0.984	–	1.923	0.1020	0.03	0.1044	0.4512
TRIS	8	8.54	0.3400	0.984	–	1.934	0.1020	0.03	0.1046	0.4512
TRIS	9	8.54	0.7725	0.984	0.0151	1.934	0.1033	0.03	0.2347	0.4512
TRIS	10	8.54	0.7045	0.984	0.0138	1.934	0.1031	0.03	0.2137	0.4534
TRIS	11	8.54	0.3921	0.984	–	1.934	0.1022	0.03	0.1201	0.4534
TRIS	12	8.54	0.3921	0.984	–	1.934	0.1022	0.03	0.1197	0.4534
TRIS	13	8.54	0.2682 [b]	0.984	–	1.934	0.1018	0.03	0.0829	0.4534
TRIS	14	8.54	0.2682 [b]	0.984	–	1.934	0.1018	0.03	0.0836	0.4534
TRIS	15	8.54	0.5463	1.483	–	1.934	0.1026	0.03	0.1718	0.4561

[a] Concentration units mM is mmol dm^{-3}, M is mol dm^{-3}.
[b] This ADP solution was further purified by ion exchange chromatography

Table A2
Concentrations of complexed and protonated species before and after the calorimetric reaction. Buffer systems HEPES and TEA

Buffer	Expt. No	ADP^{3-} (mM) [a]	$HADP^{2-}$ (μM)	$MgADP^{-}$ (mM)	$KADP^{2-}$ (mM)	ATP^{4-} (mM)	$HATP^{3-}$ (μM)	$MgATP^{2-}$ (mM)	$KATP^{3-}$ (mM)	PEP^{3-} (mM)	$HPEP^{2-}$ (μM)	$MgPEP^{-}$ (mM)	$KPEP^{2-}$ (mM)
HEPES	1–3	0.0932	5.496	0.3716	0.2807	0.0003	0.0262	0.0135	0.0007	0.3846	8.224	0.1250	0.4639
		–	–	–	–	0.0168	1.740	0.7023	0.0446	0.0932	1.994	0.0203	0.1153
HEPES	4–5	0.0814	4.794	0.3338	0.2445	0.0001	0.0102	0.0054	0.0003	0.3846	8.224	0.1250	0.4639
		–	–	–	–	0.0139	1.441	0.6180	0.0368	0.1277	2.730	0.0295	0.1575
HEPES	6–7	0.0639	3.766	0.2722	0.1916	0.0001	0.0098	0.0054	0.0003	0.3846	8.224	0.1250	0.4639
		–	–	–	–	0.0137	1.075	0.4985	0.0273	0.1802	3.853	0.0450	0.2213
TEA	1	0.0839	4.431	0.3358	0.2668	–	–	–	–	0.3876	7.558	0.1258	0.4675
		–	–	–	–	0.0146	0.0137	0.6334	0.0416	0.1198	2.337	0.0274	0.1479
TEA	2–3	0.0739	3.903	0.3020	0.2347	–	–	–	–	0.3876	7.558	0.1258	0.4675
		–	–	–	–	0.0124	1.170	0.5656	0.0354	0.1501	2.928	0.0359	0.1849
TEA	4	0.0355	1.875	0.1579	0.1123	–	–	–	–	0.3876	7.558	0.1258	0.4675
		–	–	–	–	0.0053	0.5025	0.2866	0.0150	0.2702	5.268	0.0762	0.3293
TEA	5–7	0.0406	2.140	0.1782	0.1281	–	–	–	–	0.3876	7.558	0.1258	0.4675
		–	–	–	–	0.0062	0.5813	0.3248	0.0174	0.2541	4.956	0.0702	0.3101

[a] Concentration units mM is mmol dm^{-3}, μM is micromol dm^{-3}

Table A3
Concentration of complexed and protonated species before and after the calorimetric reaction. Buffer system TRIS

Buffer pH	Expt. No	ADP^{3-} (mM) [a]	$HADP^{2-}$ (μM)	$MgADP^{-}$ (mM)	$KADP^{2-}$ (mM)	ATP^{4-} (mM)	$HATP^{3-}$ (μM)	$MgATP^{2-}$ (mM)	$KATP^{3-}$ (mM)	PEP^{3-} (mM)	$HPEP^{2-}$ (μM)	$MgPEP^{-}$ (mM)	$KPEP^{2-}$ (mM)
8.00	1	0.0920	5.623	0.3664	0.2842	–	–	–	–	0.3925	8.787	0.1276	0.4734
		–	–	–	–	0.0163	1.760	0.6855	0.0447	0.1024	2.293	0.0226	0.1267
8.00	2	0.0449	2.739	0.1969	0.1376	0.0001	0.0133	0.0073	0.0003	0.3925	8.787	0.1276	0.4734
		–	–	–	–	0.0070	0.7593	0.3630	0.0191	0.2466	5.521	0.0668	0.3013
8.00	3	0.0564	3.442	0.2409	0.1732	0.0002	0.0169	0.0091	0.0004	0.3925	8.787	0.1276	0.4734
		–	–	–	–	0.0091	0.9862	0.4486	0.0248	0.2109	4.721	0.0543	0.2585
8.00	4	0.0887	5.356	0.3498	0.2706	0.0003	0.0271	0.0135	0.0007	0.3925	8.787	0.1276	0.4734
		–	–	–	–	0.0157	1.693	0.6675	0.0430	0.1165	2.607	0.0260	0.1439
8.00	5	0.0308	1.883	0.1400	0.0944	0.0001	0.0087	0.0049	0.0002	0.3855	8.631	0.1255	0.4650
		–	–	–	–	0.0046	0.5014	0.2546	0.0125	0.2839	6.357	0.0817	0.3456
8.00	6	0.0983	6.006	0.3835	0.3037	0.0003	0.0297	0.0145	0.0008	0.3855	8.631	0.1255	0.4650
		–	–	–	–	0.0182	1.964	0.7369	0.0500	0.0780	1.747	0.0166	0.0966
8.54	7–8	0.0394	0.6854	0.1734	0.1265	–	–	–	–	0.3875	2.499	0.1261	0.4674
		–	–	–	–	0.0060	0.1859	0.3166	0.0172	0.2570	1.657	0.0715	0.3135
8.54	9	0.0949	1.653	0.3685	0.3074	0.0003	0.0082	0.0141	0.0008	0.3875	2.499	0.1261	0.4674
		–	–	–	–	0.0175	0.5447	0.7184	0.0512	0.0858	0.5535	0.0185	0.1062
8.54	10	0.0858	1.494	0.3396	0.2776	0.0002	0.0074	0.0129	0.0007	0.3877	2.500	0.1262	0.4677
		–	–	–	–	0.0153	0.4774	0.6578	0.0447	0.1134	0.7310	0.0255	0.1400
8.54	11–12	0.0457	0.7960	0.1985	0.1471	–	–	–	–	0.3877	2.500	0.1262	0.4677
		–	–	–	–	0.0071	0.2197	0.3645	0.0204	0.2368	1.527	0.0642	0.2895
8.54	13–14	0.0308	0.5355	0.1382	0.0987	–	–	–	–	0.3877	2.500	0.1262	0.4677
		–	–	–	–	0.0456	0.1419	0.2504	0.0131	0.2850	1.838	0.0822	0.3469
8.54	15	0.0650	1.132	0.2705	0.2097	–	–	–	–	0.5858	3.778	0.1845	0.7085
		–	–	–	–	0.0110	0.3410	0.5033	0.0317	0.3777	2.435	0.0911	0.4651

[a] Concentration units mM is mmol dm^{-3}, μM is micromol dm^{-3}.

Table A4
Heat fluxes associated with changes in complex formation

Buffer	Expt. No	$\dot{Q}_{MgADP}$ (mW)	$\dot{Q}_{MgATP}$ (mW)	$\dot{Q}_{MgPEP}$ (mW)	$\dot{Q}_{obs}$ (mW)	$\dot{Q}_{corr}$ (mW)	ΔH_{corr} (kJ mol^{-1})
HEPES	1	−0.0624	0.0828	−0.0086	0.0920	0.0804	14.1_1
HEPES	2	−0.0624	0.0828	−0.0086	0.0933	0.0818	14.3_6
HEPES	3	−0.0624	0.0828	−0.0086	0.0932	0.0819	14.3_7
HEPES	4	−0.0560	0.0736	−0.0078	0.0833	0.0741	14.6_9
HEPES	5	−0.0560	0.0736	−0.0078	0.0825	0.0734	14.5_5
HEPES	6	−0.0457	0.0593	−0.0066	0.0648	0.0584	14.4_7
HEPES	7	−0.0457	0.0593	−0.0066	0.0667	0.0604	14.9_6
							$14.5_0 \pm 0.3$
TEA	1	−0.0561	0.0772	−0.0081	0.1485	0.1357	25.8_4
TEA	2	−0.0505	0.0689	−0.0074	0.1338	0.1229	26.3_1
TEA	3	−0.0505	0.0689	−0.0074	0.1333	0.1224	26.2_1
TEA	4	−0.0264	0.0349	−0.0041	0.0675	0.0631	27.0_1
TEA	5	−0.0298	0.0396	−0.0046	0.0745	0.0694	26.1_5
TEA	6	−0.0298	0.0396	−0.0046	0.0755	0.0704	26.5_3
TEA	7	−0.0298	0.0396	−0.0046	0.0755	0.0704	26.5_3
							$26.3_7 \pm 0.4$
TRIS [a]	1	−0.0647	0.0874	−0.0091	0.2410	0.2277	38.0_1
TRIS	2	−0.0348	0.0453	−0.0051	0.1195	0.1143	37.3_7
TRIS	3	−0.0425	0.0560	−0.0061	0.1525	0.1455	38.3_6
TRIS	4	−0.0617	0.0834	−0.0088	0.2250	0.2124	37.2_0
TRIS	5	−0.0226	0.0292	−0.0035	0.0797	0.0767	39.1_9
TRIS	6	−0.0620	0.0844	−0.0086	0.2395	0.2261	38.9_4
							$38.1_8 \pm 0.8$
TRIS [b]	7	−0.0286	0.0383	−0.0044	0.1044	0.0992	38.7_9
TRIS	8	−0.0286	0.0383	−0.0044	0.1046	0.0994	38.8_7
TRIS	9	−0.0608	0.0852	−0.0087	0.2347	0.2191	37.7_2
TRIS	10	−0.0563	0.0784	−0.0082	0.2137	0.1999	37.5_5
TRIS	11	−0.0329	0.0443	−0.0051	0.1201	0.1138	38.4_1
TRIS	12	−0.0329	0.0443	−0.0051	0.1197	0.1134	38.2_7
TRIS	13	−0.0229	0.0304	−0.0036	0.0829	0.0790	38.9_8
TRIS	14	−0.0229	0.0304	−0.0036	0.0836	0.0797	39.3_2
TRIS	15	−0.0451	0.0615	−0.0077	0.1718	0.1631	39.2_8
							$38.5_8 \pm 0.4$

[a] Experiment numbers 1–6, pH = 8.00.
[b] Experiment numbers 7–15, pH = 8.54.

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