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THERMODYNAMICS OF HEXOKINASE-CATALYZED REACTIONS [1]

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The enthalpies of the *hexokinase*-catalyzed phosphorylation of glucose, mannose, and fructose by ATP to the respective hexose 6-phosphates have been measured calorimetrically in TRIS/TRIS · HCl buffer at 25.0, 28.5, and 32.0°C. The effects on the measured enthalpy of the glucose/*hexokinase* reaction due to variation of pH (over the range 6.7 to 9.0) and ionic strength (over the range 0.02 to 0.25) have been examined. Correction for enthalpy of buffer protonation leads to ΔH° and ΔC_p° values for the processes: eq-D-hexose + ATP^{4-} = eq-D-hexose 6-phosphate^{2-} + ADP^{3-} + H^+. Results are $\Delta H^\circ = -23.8 \pm 0.7$ kJ·mol^{-1} and $\Delta C_p^\circ = -156 \pm 280$ J·mol^{-1}·K^{-1} for glucose, $\Delta H^\circ = -21.9 \pm 0.7$ kJ·mol^{-1} and $\Delta C_p^\circ = 10 \pm 140$ J·mol^{-1}·K^{-1} for mannose, and $\Delta H^\circ = -15.0 \pm 0.9$ kJ·mol^{-1} and $\Delta C_p^\circ = -41 \pm 160$ J·mol^{-1}·K^{-1} for fructose. Combination of these measured enthalpies with Gibbs energy data for hydrolysis of ATP^{4-} and that for the hexose 6-phosphates lead to ΔS° values for the above *hexokinase*-catalyzed reactions.

1. Introduction

The Gibbs energy change (ΔG) of the *hexokinase*-catalyzed phosphorylation of glucose by ATP * to glucose 6-phosphate and adenosine 5′-diphosphate has been measured by Robbins and Boyer [1] and may also be calculated via thermochemical cycle type calculations. Thus while at present there is a substantial body of Gibbs energy data for enzyme-catalyzed processes [2,3,4], there is contrastingly little information [5] on the enthalpy (ΔH) and entropy (ΔS) changes accompanying these reactions. In addition, even for those reactions where enthalpy data do exist, to date there appear to be few systematic investigations as to the effects of variations of the relevant experimental parameters (e.g. pH, ionic strength, etc.), or as to the possible sources of systematic error that may occur in such measurements. In view of these facts and because of the importance of *hexokinase*-catalyzed reactions in intermediary metabolism, this investigation was undertaken to gain a more complete understanding of the thermodynamics of these processes. Interest in a knowledge of the enthalpy changes of enzyme-catalyzed processes also stems from: (1) recent work on the use of heat measurements for bioanalytical purposes [6–8], (2) possible applications to the field of muscle physiology [9], and (3) relevancy to biochemical thermodynamics [10].

2. Materials and methods

2.1. Materials

Reagents and their sources ‡ were: crystalline D-glucose, National Bureau of Standards, Standard Reference Material No. 917; crystalline D-mannose,

[1] This work was presented, in part, before the Minneapolis, Minnesota meeting of the Biophysical Society, June 3, 1974.

* Abbreviations used in this paper are: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; TRIS, tris(hydroxymethyl)aminomethane; G6P, glucose 6-phosphate; M6P, mannose 6-phosphate; F6P, fructose 6-phosphate; H6P, hexose 6-phosphate; P_i, inorganic phosphate.

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

samples from Pfanstiehl Laboratories, Inc. and Calbiochem; crystalline D-fructose, samples from Pfanstiehl Laboratories, Inc. and Calbiochem; lyophilized yeast *hexokinase*, Calbiochem (two different lots) and Worthington Biochemical Corporation; magnesium chloride hexahydrate, Allied Chemical Co.; TRIS, Eastman Organic Chemicals and Fisher Scientific Company (Fisher certified primary standard); concentrated hydrochloric acid, Mallinckrodt Chemical Co. and Allied Chemical Co.; di-sodium salt of ATP, Calbiochem and Sigma Chemical Co.; di-TRIS salt of ATP, Sigma Chemical Co.; distilled water, stock supply at the National Bureau of Standards.

The D-glucose is a well characterized material, having a certified [11] purity of 99.9 percent. Additional information provided by the suppliers on the characterization of the other reagents is as follows: Pfanstiehl D-mannose, purity 99+%, specific rotation $+14.2 \pm 0.4°$ at 20°C, residue after ignition less than 0.05%, moisture content 0.1%; Calbiochem D-mannose, specific rotation of 14.3° at 22°C, chromatographically homogeneous in three different solvent systems; Pfanstiehl D-fructose, purity 99+%, specific rotation −92.1°, residue after ignition 0.05%, moisture content of less than 0.1%; *hexokinase*, contaminating activities are *glutathione reductase* (Calbiochem lot A, 0.001%, Calbiochem lot B, 0.02%, Worthington, 0.02%), *phosphoglucose isomerase* (Calbiochem lot A, 0.007%, Calbiochem lot B, 0.035%, Worthington, none detectable), *adenylate kinase* (Calbiochem lot A, 0.001%; Calbiochem lot B, 0.0003%; Worthington, negligible), *6-phosphogluconic dehydrogenase* (Calbiochem lot A, 0.001%, Calbiochem lot B, negligible, Worthington, negligible), and *glucose 6-phosphate dehydrogenase* (Calbiochem lot A, 0.004%, Calbiochem lot B, 0.0038%, Worthington, 0.06%); di-sodium salt of ATP (Calbiochem), contaminants are inorganic phosphate 0.16% and calcium 0.9%; di-sodium salt of ATP (Sigma), purity of 99% when prepared, major impurity is ADP; di-TRIS salt of ATP (Sigma), purity of 97% when prepared, sodium content 0.3%, major impurity is ADP.

Analysis for moisture content on the hexose samples was performed by the Karl Fischer method. Results indicated that in all cases moisture content was less than 0.2% and no corrections for moisture are applied to the final results.

Analysis for total magnesium in several of the samples was kindly performed by T.C. Rains of the Analytical Chemistry Division of the National Bureau of Standards by means of atomic-absorption spectrometry. The results of these analyses, expressed as μg magnesium per gram of sample, were as follows: TRIS (Eastman), 0.12; di-sodium salt of ATP (Calbiochem), 5.5; *hexokinase* (Calbiochem lot A), 3500; D-glucose (NBS), 0.37; Pfanstiehl D-mannose, 10.5; and Pfanstiehl D-fructose, 0.05.

Polarimetric measurements were performed in order to determine that in solution the equilibrium mixture of aqueous hexoses was present. The results of the measurements showed that in TRIS/TRIS·HCl buffer at pH 8.7, the mutarotation processes were essentially complete within $1\frac{1}{2}$ h following the solution of the respective hexoses in the buffer.

2.2. *Calorimetric measurements*

Heat measurements were performed using a heat-conduction microcalorimeter similar to one previously described [12] in which two separate solutions, designated as enzyme solution and substrate solution, respectively, are mixed by rotation of the calorimetric heat sink. The heat sink accommodates a single bicompartmental reaction vessel (≈ 2 ml total volume) which is constructed of high-density polyethylene. The calibration constant of the instrument used in this investigation has been measured by direct electrical calibrations, including heater-placement tests, repeated at periodic intervals before, during, and after this investigation was concluded, and is $17.57\ W \cdot V^{-1}$ at 25°C. The imprecision of reaction-heat measurement, as established by measurements of the enthalpy of reaction of hydrochloric acid with excess sodium hydroxide has been found to be as good as 0.07% (estimated standard deviation of a measurement) at a total heat input of ≈900 mJ for a rapid chemical reaction. Total inaccuracy of heat measurement is estimated to be no greater than 0.3%. The half-response time of the instrument is in the range 60 to 90 sec. The amplified thermopile voltage was recorded in digital form and the peak area associated with the chemical-reaction energy was calculated by numerical integration of the time—voltage data. The temperature of reaction was determined with either an NBS-calibrated platinum resistance thermometer or a copper/constantan thermocouple referenced against an ice—water mixture, and the

measured heat refers to the isothermal change in internal energy (ΔU) of the system at the specified temperature. Since the reactions being studied in this investigation occur in the condensed phase, the difference between ΔU and ΔH will be relatively small [13] and hence will be neglected.

2.3. Preparation of solutions

The substrate solution consisted of gravimetrically measured quantities of hexose (the limiting reactant), ATP, $MgCl_2$ (in a few experiments), and aqueous TRIS/TRIS·HCl buffer adjusted with HCl to a known pH value. The enzyme solution was prepared by pre-reacting a portion of the previously prepared substrate solution with a known quantity of lyophilized *hexokinase*. With the exception of only one experiment (appendix, table A2, no. 34), all heat measurements were completed within a period varying from 100 min to 8 h following preparation of the solutions.

Measurements of the pH of the reacting solutions were performed using a Corning Model 12 pH meter with a Fisher Scientific Co. glass/calomel combination micro-electrode, the system being calibrated using standard buffers at pH 7.00 (Fisher-certified buffer), 7.41 (NBS-certified buffer [14]), and 8.00 (Fisher-certified buffer). All pH measurements reported herein are judged to be accurate to within ±0.03 pH units.

2.4. Calculations

The molar enthalpy of reaction (ΔH_{obs}) is taken as the quantity $-Q_{meas}/n$, where Q_{meas} is the measured heat of reaction under the specified set of conditions and n is the number of moles of hexose present in the substrate solution. Relative molecular masses are based on the 1969 scale [15].

Calculation of ionic strength (I) was performed by considering contributions from the ions ATP^{4-}, $HATP^{3-}$, $TRIS \cdot H^+$, Na^+, and Cl^- with the usual relationship $I = \frac{1}{2}\sum_i m_i z_i^2$ where m_i is the molality of the ith ion and z_i is its signed charge.

Statistical measures of imprecision used in this paper are computed according to the relationships given in ref. [16]. All tests of significance applied herein refer to the uncertainty interval equal to twice the estimated standard deviation of the mean, which is approximately at the 95% confidence limit. Least-squares computations were performed using OMNITAB statistical procedures [17].

3. Results and discussion

3.1. Specification of the reactions occurring

The reaction of a D-hexose with ATP may be represented as

$$\text{eq-D-hexose} + \sum\text{ATP} = \sum\text{eq-D-H6P} + \sum\text{ADP} + n_H H^+ , \quad \text{(A)}$$

where the symbol Σ indicates that there may exist different ionic and metal bound states (e.g. ATP^{4-}, $HATP^{3-}$, $MgATP^{2-}$, $NaATP^{3-}$, etc.), and n_H is the number of protons liberated in the reaction. The hexoses are taken to be present in their equilibrium conformational mixtures (designated by the symbol 'eq' above). For aqueous glucose and mannose only the α and β forms need be considered present, while for fructose there is also present a furanose/pyranose equilibrium. It is understood that the reaction is occurring in an aqueous medium at concentrations and ionic strengths to be specified for each experiment performed.

Reactions of the above type involving coupled equilibria are characteristic of many biochemical processes and formalisms suitable for their treatment have previously been applied [18–20] to the hydrolysis reaction of ATP:

$$\sum\text{ATP} + H_2O = \sum\text{ADP} + \sum P_i + n_H H^+ . \quad \text{(B)}$$

Since reaction (A) is very similar to (B) it is possible to apply previously derived equations with only minor modifications. Hence, in the absence of metal ions binding to the reactants and products, and following the notation of Alberty [19], ΔH_A° is given by:

$$\begin{aligned}\Delta H_A^\circ = {} & \Delta H_c^\circ \\ & - f_{ADP}\{[(H^+)/K_{1ADP}]\Delta H_{1ADP}^\circ + [(H^+)^2/K_{1ADP}K_{2ADP}] \\ & \times (\Delta H_{1ADP}^\circ + \Delta H_{2ADP}^\circ)\} - f_{H6P}\{[(H^+)/K_{H6P}]\Delta H_{H6P}^\circ\} \\ & + f_{ATP}\{[(H^+)/K_{1ATP}]\Delta H_{1ATP}^\circ \\ & + [(H^+)^2/K_{1ATP}K_{2ATP}](\Delta H_{1ATP}^\circ + \Delta H_{2ATP}^\circ)\} . \quad \text{(1)}\end{aligned}$$

ΔH_c° is the molar enthalpy change under standard-state conditions for the reference reaction involving specific ionic forms:

$$\text{eq-D-hexose} + \text{ATP}^{4-} = \text{eq-D-H6P}^{2-} + \text{ADP}^{3-} + \text{H}^+ . \quad \text{(C)}$$

In this investigation we have taken our standard state to be the hypothetical ideal solution of unit molality, which for the enthalpy of reaction corresponds to that enthalpy which would be measured in the infinitely dilute real solution. The fractions of ATP, ADP, and H6P existing in the most basic forms are given by

$$f_{\text{ATP}} = (\text{ATP}^{4-})/\textstyle\sum\text{ATP} = [1 + \{(\text{H}^+)/K_{1\text{ATP}}\}\{1 + (\text{H}^+)/K_{2\text{ATP}}\}]^{-1}, \quad (2)$$

$$f_{\text{ADP}} = (\text{ADP}^{3-})/\textstyle\sum\text{ADP} = [1 + \{(\text{H}^+)/K_{1\text{ADP}}\}\{1 + (\text{H}^+)/K_{2\text{ADP}}\}]^{-1}, \quad (3)$$

and

$$f_{\text{H6P}} = (\text{H6P}^{2-})/\textstyle\sum\text{eq-D-H6P} = [1 + (\text{H}^+)/K_{\text{H6P}}]^{-1}. \quad (4)$$

The number of protons produced is

$$n_{\text{H}} = 1 - [(\text{H}^+)/K_{1\text{ADP}} + 2(\text{H}^+)^2/K_{1\text{ADP}}K_{2\text{ADP}}]f_{\text{ADP}} - [(\text{H}^+)/K_{\text{H6P}}]f_{\text{H6P}} + [(\text{H}^+)/K_{1\text{ATP}} + 2(\text{H}^+)^2/K_{1\text{ATP}}K_{2\text{ATP}}]f_{\text{ATP}}. \quad (5)$$

The molar-enthalpy change measured when the reaction occurs in a buffered medium is given by

$$\Delta H_{\text{obs}}^\circ = \Delta H_{\text{A}}^\circ + n_{\text{H}}\,\Delta H_{\text{D}}^\circ , \quad (6)$$

where $\Delta H_{\text{A}}^\circ$ is the molar enthalpy of reaction for process (A) in the absence of any buffer protonation processes and $\Delta H_{\text{D}}^\circ$ is the enthalpy change for the buffer protonation reaction, e.g.

$$\text{TRIS} + \text{H}^+ = \text{TRIS}\cdot\text{H}^+ . \quad \text{(D)}$$

Since ΔH_{obs}° will vary with pH and metal-ion concentration (which makes for even more complex equations), if one wishes to obtain a reliable value for ΔH_c°, it is best to perform measurements under conditions where $\Delta H_{\text{obs}}^\circ$ does not vary with pH (i.e. in a pH range well separated from the pK values of the reactants and products, thus eliminating the necessity for ionization corrections) and with only trace quantities of magnesium present to insure sufficient enzymatic activity. The presence of a buffer is also desirable in order to define adequately the ionic states of the reactants and products. However, it does require a large correction to be made for the enthalpy of buffer protonation, which is nevertheless well known.

Both, equilibrium measurements [1] and quenching experiments [8], show that the reactions studied in this investigation proceed essentially to completion.

3.2. Experimental data

The experimental data are presented in the appendix as follows: table A1, 'blank' heat effects; table A2, data for the glucose/*hexokinase* reaction at various pH values from 6.7 to 9.0 (see also fig. 1); table A3, data for the glucose/*hexokinase* reaction as a function of ionic strength (see also fig. 2); table A4, data for the

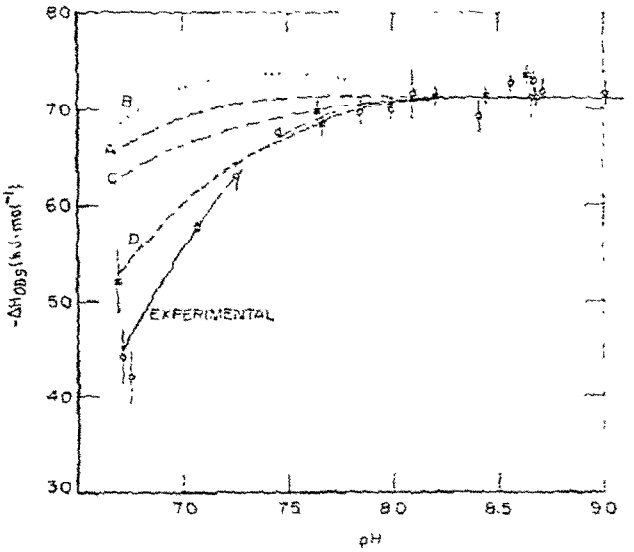

Fig. 1. Plot of ΔH_{obs} for the glucose/*hexokinase* reaction in TRIS/TRIS·HCl buffer as a function of pH. Ionic strength is from 0.093 to 0.477. Temperature = 25.0°C. Half the length of a drawn error bar is twice the estimated standard deviation of the mean for replicate measurements made at the same pH (indicated in the figure by $\dot{\varphi}$). For individual measurements (indicated by $\dot{\varphi}$) half the length of a drawn error bar is twice the pooled standard deviation calculated from replicate measurements performed in the same pH range. Also shown are four curves calculated using eqs. (1–5). Curve A is obtained using the data given in the text (see section on ΔH_{obs} as a function of pH). The other curves are obtained by changing only one pK value, namely, curve B is obtained using $pK_{1\text{ATP}} = 7.05$; curve C, $pK_{1\text{ADP}} = 6.98$; and for curve D, $pK_{\text{H6P}} = 6.60$.

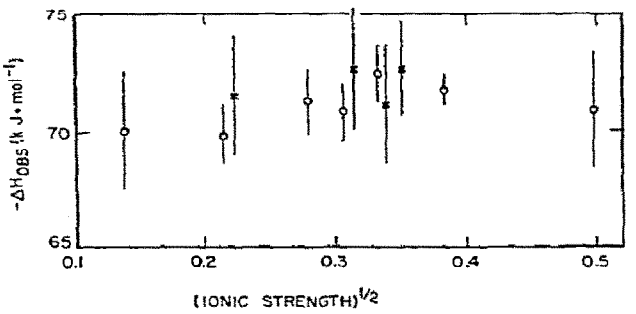

Fig. 2. Plot of ΔH_{obs} for the glucose/*hexokinase* reaction in TRIS/TRIS·HCl buffer as a function of the square root of the computed ionic strength. pH is from 8.64 to 8.72. Included in the figure are data from the appendix, table A3 that are in this same pH range (experiments nos. 23–46). The length of the error bars drawn in this figure is based on the same scheme as that used in fig. 1.

glucose/*hexokinase* reaction using the di-TRIS salt of ATP; table A5, data for the mannose/*hexokinase* reaction; table A6, data for the fructose/*hexokinase* reaction; table A7, data for the glucose/*hexokinase*, mannose/*hexokinase*, and fructose/*hexokinase* reactions at 28.5 and 32.0°C. We have been detailed in the presentation of experimental results so that if additional information bearing on their interpretation later becomes available, the data may be reevaluated.

3.3. *'Blank' heat effects*

The average of four heat measurements involving zero reacting hexose is 0.24 mJ with an estimated standard deviation of the mean of 1.61 mJ. Since the average does not vary significantly from zero, we apply no correction for 'blank' heat effects to our measured reaction enthalpies.

3.4. ΔH_{obs} *as a function of pH*

The results of measurements of ΔH_{obs} as a function of pH are shown in fig. 1. If in the pH range 8.0 to 9.0, the variation of ΔH_{obs} with pH is assumed to fit the equation ΔH_{obs} (kJ · mol^{-1}) = $A + B$ (pH), where A and B are coefficients to be obtained by statistical procedures, least-squares computation of A and B (using experiments no.17 to 51 in the appendix, table A2) yields the following results: $A = -57.30$ and $B = -1.64$ (the estimated standard deviations of these coefficients are 7.6 and 0.89, respectively). Therefore, in the pH range 8.0 to 9.0, ΔH_{obs} may be considered to be a constant within the imprecision of our measurements and is 71.34 kJ · mol^{-1} (estimated standard deviation of the mean is 0.26 kJ · mol^{-1}). Hence, we feel confident that in this pH range, ΔH_c may be obtained from ΔH_{obs} without the use of correction terms calculated using eqs. (1–5) and the Gibbs energies and enthalpies of ionization of the reactants and products.

To calculate the variation of ΔH_{obs} with pH we have used eqs. (1–5) with the following data: $pK_{1ATP} = 6.95$, $pK_{2ATP} = 4.06$, $pK_{1ADP} = 6.86$, $pK_{2ADP} = 3.93$, $\Delta H^\circ_{1ATP} = -7.03$ kJ · mol^{-1}, $\Delta H^\circ_{2ATP} = 0.0$ kJ · mol^{-1}, $\Delta H^\circ_{1ADP} = -5.73$ kJ · mol^{-1}, and $\Delta H^\circ_{2ADP} = 4.18$ kJ · mol^{-1} from ref. [19]; $pK_{H6P} = 6.03$ [21]; an estimated $\Delta H^\circ_{H6P} = 0.0$ kJ · mol^{-1}; $\Delta H^\circ_c = -23.8$ kJ · mol^{-1} (obtained later in this paper); and $\Delta H^\circ_D = -47.48$ kJ · mol^{-1} [22]. The result obtained using these input data is shown in fig. 1 (curve A). Since the above data may have inaccuracies associated with them, it is of interest to generate other curves by variation of the above input data, and these curves are also shown in fig. 1. The calculated variation of ΔH_{obs} with pH is seen not to agree with the experimental data at the lower pH values. If we assume that the experimental data are correct, this discrepancy may be accounted for by either the input data possessing larger systematic errors than one should expect, or, possibly, neglect of additional terms in eqs. (1–5) for sodium-binding or ionic-strength effects. We note that the results of this computation are most sensitive to the values selected for the quantities pK_{1ATP}, pK_{1ADP}, and pK_{H6P}. This sensitivity is attributable to the fact that the quantity $n_H \Delta H^\circ_D$ is determined almost entirely by these pK values, and it, in turn, makes the largest single contribution to ΔH_{obs}. While a resolution of these discrepancies is of some interest, it is of only tangential importance to the determination of accurate values for ΔH°_c. The results of the computation do also show, however, that the ionization corrections to be applied to ΔH_{obs}, in order to obtain an accurate value for ΔH°_c, are negligible at pH values greater than 8.0.

3.5. ΔH_{obs} *as a function of ionic strength*

Fig. 2 is a plot of ΔH_{obs} as a function of the square

root of the computed ionic strength, the pH range being from 8.64 to 8.72. If we assume that for this data ΔH_{obs} (kJ · mol^{-1}) = $C + D(I)^{1/2}$, least-squares computation of C and D (using experiments no. 31 to 46 in the appendix, table A2, and all of the experiments in the appendix, table A3) yield $C = -69.72$ and $D = -4.84$ (estimated standard deviations of these coefficients are 0.93 and 2.9, respectively). Since D does not vary significantly from zero we conclude that, within our experimental imprecision, ΔH_{obs} does not vary over the range of ionic strength investigated. We, therefore, average these results and take ΔH_{obs} equal to −71.19 kJ · mol^{-1} (estimated standard deviation of the mean equal to 0.30 kJ · mol^{-1}) over the range of ionic strength investigated.

Available information [23] on the heats of dilution of electrolyte solutions having the same charge type as the reactants and products may be used to estimate the magnitude of the dilution correction from $I = 0.01$ to $I = 0$. Doing this, an estimated correction to infinite dilution of ≈ +0.4 kJ · mol^{-1} is obtained. However, in the absence of experimental data on the actual materials, we choose to make no correction for the heats of dilution, but nevertheless associate our ΔH_{obs} with ΔH°_{obs}. Should these data later become available, our results are presented in sufficient detail that the proper corrections may be applied.

3.6. di-TRIS ATP measurements

The experiments summarized above were all performed using the di-sodium salt of ATP and with only catalytic quantities of magnesium present. Hence, there exists the possibility that ΔH°_{c} may have been affected by sodium binding [24] to the reactants and products. Therefore, some experiments were performed (see appendix, table A4) using the di-TRIS salt of ATP. Since the average of these results −70.87 kJ · mol^{-1} (estimated standard deviation of the mean of 0.52 kJ · mol^{-1}) does not differ significantly from the above results, we conclude that the effect of sodium binding to the reactants and products is not a large one and no serious error is incurred in extracting a value of ΔH°_{c} from our average ΔH°_{obs} obtained in the pH range 8.0 to 9.0 and using the di-sodium salt of ATP.

3.7. Mannose and fructose results

The mannose and fructose results are shown in the appendix, tables A5 and A6, respectively. Note that in each case two different samples of hexose were used as the limiting reactant. Since no significant differences between the different samples was calculated, we average the six results to obtain ΔH_{obs}(mannose) = −69.32 kJ · mol^{-1} and ΔH_{obs}(fructose) = −62.45 kJ · mol^{-1} with estimated standard deviations of the mean being 0.35 and 0.43 kJ · mol^{-1}, respectively. As in the case of the glucose results, we take ΔH_{obs} equal to ΔH°_{obs}. Since the pK value for F6P is very nearly identical to that for G6P [21] and since experiments were performed at pH = 8.6, no ionization corrections need be applied to the results obtained for the fructose/*hexokinase* reaction. On the basis of structural similarity, we judge the same to be true for the mannose/*hexokinase* reaction.

3.8. Buffer protonation correction

Values of ΔH°_{c} for glucose, mannose, and fructose are obtained by application of eq. (6) with n_H taken equal to unity. For ΔH°_{D} we use the value of −47.48 ± 0.03 kJ · mol^{-1} from Öjelund and Wadsö [22]. This value is in excellent agreement with the value of −47.46 kJ · mol^{-1} that may be calculated from the results of Prosen and Kilday [25]. ΔH_D should show only negligible dependence on ionic strength [22]. Combination of our experimental results with the above value for ΔH°_{D} yields the following: ΔH°_{c} (glucose) = −23.8 kJ · mol^{-1}, ΔH°_{c} (mannose) = −21.9 kJ · mol^{-1}, and ΔH°_{c} (fructose) = −15.0 kJ · mol^{-1}.

3.9. ΔC°_{p} for the hexokinase reactions

Results of measurements performed at temperatures higher than 25°C are given in the appendix, table A7. Measurements were also attempted at 35 and 37°C, but loss of enzymatic activity at these temperatures precluded measurements with any degree of precision. Least-squares computation of ΔC°_{p} from the measured data and the usual relationship $\Delta C^{\circ}_{p} = (\partial \Delta H^{\circ}/\partial T)_p$ yields the following results (all in TRIS/TRIS-HCl buffer): $\Delta C^{\circ}_{p} = -206 \pm 280$ J · mol^{-1} · K^{-1} for the glucose/*hexokinase* reaction; $\Delta C^{\circ}_{p} = 60 \pm 40$

$J \cdot mol^{-1} \cdot K^{-1}$ for the mannose/*hexokinase* reaction; and $\Delta C_p^\circ = -91 \pm 160\ J \cdot mol^{-1} \cdot K^{-1}$ for the fructose/*hexokinase* reaction. The stated uncertainties are twice the estimated standard deviations of the coefficients obtained from the least-squares computation.

ΔC_p° values for the processes (C) are calculated by using the value $\Delta H_D^\circ = -47.48\ kJ \cdot mol^{-1}$ at 25.0°C [22] and $\Delta C_p^\circ = 50 \pm 5\ J \cdot mol^{-1} \cdot K^{-1}$ for process (D) which is calculated from the data given by Prosen and Kilday [26]. The ΔC_p° values calculated for the processes (C) are: $\Delta C_p^\circ = -156 \pm 280\ J \cdot mol^{-1} \cdot K^{-1}$ (glucose/*hexokinase*); $\Delta C_p^\circ = 10 \pm 140\ J \cdot mol^{-1} \cdot K^{-1}$ (mannose/*hexokinase*); and $\Delta C_p^\circ = -41 \pm 160\ J \cdot mol^{-1} \cdot K^{-1}$ (fructose/*hexokinase*).

3.10. Imprecision and inaccuracy of measurements

We note that the imprecision of our enzymatic heat measurements is larger than is obtained when measuring a 'clean' chemical reaction such as the neutralization of HCl with excess of NaOH. This is, in part, attributable to the slow rate of reaction observed in many experiments (some experiments lasted over three hours), particularly those at low pH values. This slow rate of reaction, in contrast to rapid rates observed in a previous investigation [7], is probably attributable to the fact that now only trace quantities of magnesium are present.

In table 1 we estimate reasonable upper limits for possible sources of systematic error in our measurements. We take our total estimate of systematic error to be the square root of the sums of the squares of the estimated sources of error given in table 1 and obtain a value of 0.7 $kJ \cdot mol^{-1}$ for the three reactions studied herein, with the largest contribution coming from the uncertainty associated with the dilution corrections. Since the estimated systematic error is within the imprecision of our measurements, we take our final assigned uncertainty to be twice the estimated standard deviations of the mean for the three hexoses studied herein.

Table 1
Judgment of possible sources of systematic error.

Source of error	Estimate of error ($kJ \cdot mol^{-1}$)
Heat-measurement error	0.21
Impurities in hexoses	
glucose	0.07
mannose and fructose	0.15
Enzymatic impurities	0.10
Ionization corrections for reactants and products	0.10
Metal-ion binding to reactants and products	0.30
Buffer protonation correction	0.10
Correction to standard state	0.50
Incomplete mutarotation	0.05
Incomplete reaction	0.01
Total:	0.7

3.11. Comparison with other measurements

Heat measurements on the glucose/*hexokinase* reaction have been performed in conjunction with analytical applications [7,8]. To compare the result of $-61.4\ kJ \cdot mol^{-1}$ (ATP to magnesium ratio of 2.5 to 1.0, pH in the range 7.1 to 7.4, temperature equal to 30.8°C) with the value of $-71.2\ kJ \cdot mol^{-1}$ (trace magnesium present, pH in the range 8.0 to 9.0, temperature equal to 25.0°C) requires correction for the effects of magnesium, pH, and temperature. We use our experimental plot of ΔH_{obs} as a function of pH (fig. 1) to obtain a correction of $-8.2 \pm 4.0\ kJ \cdot mol^{-1}$ for the effect of pH (the estimated uncertainty associated with this correction is attributable to (1) the scatter inherent in the data shown in fig. 1 and (2) uncertainty as to the precise pH of the reacting solution used in ref. [7]); enthalpies of binding of Mg^{2+} to ATP^{4-} and ADP^{3-} [26] to obtain a correction of $+1.4\ kJ \cdot mol^{-1}$ for magnesium binding; our ΔC_p° of $-206 \pm 280\ J \cdot mol^{-1} \cdot K^{-1}$ in obtaining a correction of $+1.2\ kJ \cdot mol^{-1}$ for the effect of temperature. The adjusted result of $-67 \pm 4\ kJ \cdot mol^{-1}$ is, within the given uncertainties, in agreement with the result of $-71.3 \pm 0.7\ kJ \cdot mol^{-1}$.

McGlothlin and Jordan [8] report a value of $\Delta H_{obs} = -74.9 \pm 1.5\ kJ \cdot mol^{-1}$ for the glucose/*hexokinase* reaction in TRIS/TRIS·HCl buffer (pH = 8.0, total magnesium equal to total ATP concentration, temperature equal to 25.0°C). Using enthalpies of binding of Mg^{2+} to ATP^{4-} and ADP^{3-} [26] we apply a correction of $+3.4\ kJ \cdot mol^{-1}$ to their reported value and obtain a value of $-71.5 \pm 1.5\ kJ \cdot mol^{-1}$ which is in excellent agreement with our result of $-71.3\ kJ \cdot mol^{-1}$.

Table 2
Thermodynamics of *hexokinase*-catalyzed reactions at 25.0°C. Standard state is the hypothetical ideal solution of unit molality for all reactants and products.

	ΔG° (kJ·mol^{-1})	ΔH° (kJ·mol^{-1})	ΔS° (J·mol^{-1}·K^{-1})	ΔC_p° (J·mol^{-1}·K^{-1})
eq-D-Glucose + ATP^{4-} = eq-D-G6P^{2-} + ADP^{3-} + H^+	+16.7 a)	−23.8 ± 0.7 b)	−136	−156 ± 280 b)
eq-D-Mannose + ATP^{4-} = eq-D-M6P^{2-} + ADP^{3-} + H^+	+15.7 a)	−21.9 ± 0.7 b)	−126	10 ± 140 b)
eq-D-Fructose + ATP^{4-} = eq-D-F6P^{2-} + ADP^{3-} + H^+	+18.7 a)	−15.0 ± 0.9 b)	−113	−41 ± 160 b)

a) Calculated by combination of averaged results of Guynn and Veech [28] and Benzinger et al. [29] with results of Meyerhof and Green [27].
b) This work.

3.12. Gibbs energy change and ΔS° values

Robbins and Boyer [1] obtained an experimental equilibrium quotient for the glucose/*hexokinase* reaction at pH 6.0 and applied large ionization corrections to arrive at a result of ΔG° = +5.05 kcal·mol^{-1} = +21.1 kJ·mol^{-1} at 30°C for reaction (C) involving glucose. We use this result in combination with the data of Meyerhof and Green [27] to calculate * ΔG° = +8.6 kJ·mol^{-1} for the process

$$ATP^{4-} + H_2O(liq) = ADP^{3-} + HPO_4^{2-} + H^+ . \qquad (E)$$

Recently, Guynn and Veech [28] have summarized the results of various thermodynamic pathways leading to ΔG° for the hydrolysis of ATP^{4-}. Adjusting the above ΔG° value of +8.6 kJ·mol^{-1} to the conditions used by Guynn and Veech in their table IV (T = 37°C, hypothetical one-molal standard state for all reactants and products, with the exception of the hydrogen ion which is at pH 7), we arrive at a value of −31.8 kJ·mol^{-1} which is near the lower end of their tabulated values. Because the final result of Robbins and Boyer is largely dependent on large and somewhat uncertain ionization corrections and also since we feel that an average value obtained from the investigations of Guynn and Veech [28] and Benzinger et al. [29] is presently the most reliable for process (E), we choose to use it in conjunction with the results of Meyerhof and Green [29] and calculate the ΔG° values given in table 2. ΔS° values are obtained by combination with the measured enthalpies.

* In making temperature corrections to the measured equilibrium data, the ΔH° values used were from Kitzinger and Benzinger [30] and our own measurements.

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Table A1 Experimental data for "blank heat effects". *Temperature is* 25.04°C.

Exper. No.	Materials[1]	Hexose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ
1	A1, E1, B1, H1	Zero	10.00	0.3574	8.72	0.1166	0.89	0.8268	0.5242	-2.39
2	A1, E1, B1, H1	Zero	10.00	0.3574	8.72	0.1166	0.89	0.9167	0.4781	4.73
3	A1, E1, B1, H1	Zero	10.00	0.3574	8.72	0.1166	0.89	1.0179	0.5781	0.37
4	A1, E1, B1, H1	Zero	10.00	0.3574	8.72	0.1166	0.89	0.8627	0.4024	-1.77

Average = +0.24 mJ

Estimated standard deviation of the mean = 1.61 mJ

[1] Symbols in this column designate the materials used in each experiment. The key is as follows: C1, D-glucose from NBS; C2 and C3, D-mannose from Calbiochem and Pfanstiehl, respectively; C4 and C5, D-fructose from Calbiochem and Pfanstiehl, respectively; E1 and E2, hexokinase from Calbiochem (two different lots); E3, hexokinase from Worthington; A1 and A2, di-sodium salt of ATP from Calbiochem and Sigma, respectively; A3, di-TRIS salt of ATP from Sigma; B1 and B2, TRIS from Eastman and Fisher, respectively; H1 and H2 from Allied Chemical Co. and Mallinckrodt Chemical Co., respectively.

Table A2 Experimental data for the glucose/hexokinase reaction as a function of pH. *Temperature is* 25.04°C.

Exper. No.	Materials	Glucose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of Hexose reacted, μmol	-Molar enthalpy, kJ·mol^{-1}
1*	A1, E1, C1, B1, H1	5.112	10.19	0.1801	6.69	0.211	1.17	0.7594	0.4874	202.0	3.852	52.43
2	A1, E1, C1, B2, H2	4.495	13.54	0.1811	6.71	0.227	2.265	0.7690	0.3104	256.9	3.456	43.39
3	A1, E1, C1, B2, H2	4.495	13.54	0.1811	6.71	0.227	2.265	0.7535	0.3654	244.0	3.387	42.53
4	A1, E2, C1, B2, H2	5.241	13.35	0.1811	6.75	0.477	2.317	0.7738	0.4135	163.8	4.055	40.40
5	A1, E2, C1, B2, H2	5.241	11.35	0.1803	6.75	0.477	2.317	0.7567	0.6062	171.1	1.955	43.27
6	A1, E1, C1, B2, H2	4.826	14.12	0.1831	7.06	0.229	0.971	0.7656	0.4817	212.8	3.696	57.58
7	A1, E1, C1, B2, H2	4.826	14.12	0.1831	7.06	0.229	0.971	0.7098	0.5698	220.1	3.813	57.72
8	A1, E1, C1, B2, H2	4.817	23.66	0.1835	7.15	0.223	2.00	0.7711	0.4302	239.6	3.714	62.08
9	A1, E1, C1, B2, H2	4.817	13.68	0.1835	7.13	0.223	2.00	0.7789	0.5437	239.3	3.712	63.79
10	A1, E1, C1, B1, H1	5.120	11.14	0.1818	7.45	0.200	1.47	0.7749	0.4996	269.2	3.988	67.84
11	A1, E1, C1, B2, H2	4.935	13.73	0.1832	7.46	0.214	1.94	0.7857	0.5011	262.7	3.877	67.31
12	A1, E1, C1, B2, H2	4.935	13.73	0.1832	7.46	0.214	1.94	0.7789	0.4822	259.7	3.843	67.51
13	A1, E1, C1, B2, H2	4.830	15.10	0.1828	7.64	0.211	1.44	0.7939	0.4762	267.5	3.933	69.76
14	A1, E1, C1, B1, H1	5.110	11.14	0.1818	7.67	0.185	2.29	0.7314	0.4785	259.1	3.745	69.22
15	A1, E1, C1, B2, H2	4.782	13.25	0.1830	7.44	0.185	1.97	0.7690	0.4654	264.9	3.773	70.22
16	A1, E1, C1, B2, H2	4.782	13.25	0.1830	7.44	0.183	1.97	0.6953	0.5009	229.6	3.325	69.03
17	A1, E2, C1, B2, H2	5.474	13.45	0.1807	7.49	0.168	2.56	0.7836	0.4788	296.1	4.160	70.84
18	A1, E2, C1, B2, H2	5.474	13.45	0.1807	7.49	0.168	2.56	0.7800	0.5116	296.9	4.230	70.00
19	A2, E1, C1, B1, H1	5.110	13.45	0.1818	8.00	0.155	1.43	0.8430	0.4691	297.3	4.316	68.87
20	A1, E1, C1, B2, H2	4.756	13.68	0.1834	8.09	0.161	0.89	0.7618	0.3130	204.5	3.626	72.96

Table A2 (continued)

21	A1, B1, C1, B2, H2	4.756	13.68	0.1836	8.09	0.161	0.89	0.7245	0.5123	241.9	3.449	70.14
22‡	A1, B1, C1, B1, H1	5.112	10.19	0.1801	8.20	0.130	1.61	0.7186	0.3646	265.5	3.726	71.26
23	A1, B1, C1, B1, H1	5.109	12.86	0.0897	8.41	0.101	1.33	0.9146	0.5193	330.3	4.673	70.68
24	A1, B1, C1, B1, H2	5.109	12.86	0.0897	8.41	0.101	1.33	0.8917	0.4201	308.4	4.356	67.69
25	A1, B1, C1, B1, H1	5.025	13.16	0.0876	8.42	0.102	1.39	0.7158	0.5084	244.0	3.597	67.83
26	A1, B1, C1, B1, H1	5.025	13.16	0.0876	8.42	0.102	1.39	0.8980	0.4111	318.5	4.512	70.59
27	A1, B1, C1, B1, H1	5.025	13.16	0.0876	8.42	0.102	1.39	0.8136	0.3272	297.7	4.088	72.82
28‡	A1, B1, C1, B1, H1	5.112	10.19	0.1801	8.44	0.109	0.91	0.6839	0.5459	249.7	3.696	71.42
29‡	A1, B1, C1, B1, H1	4.276	22.51	0.1790	8.56	0.172	0.50	0.8193	0.5190	256.7	3.548	72.39
30‡	A1, B1, C1, B1, H1	4.276	22.51	0.1790	8.56	0.172	1.37	0.7131	0.4891	222.7	3.049	73.04
31	A1, B1, C1, B2, H2	5.761	15.17	0.1810	8.64	0.124	1.36	0.8006	0.5192	340.1	4.612	73.74
32‡	A1, B1, C1, B1, H1	4.900	10.16	0.1803	8.66	0.093	0.37	0.6329	0.3988	225.8	3.101	72.82
33‡	A1, B1, C1, B1, H1	4.900	10.16	0.1803	8.66	0.093	0.37	0.7281	0.3876	253.7	3.568	71.10
34‡‡	A1, B1, C1, B1, H1	4.900	10.16	0.1803	8.66	0.093	2.01	0.7187	0.3053	241.0	3.522	68.43
35	A1, B1, C1, B2, H2	5.120	11.14	0.1818	8.68	0.099	2.91	0.7966	0.4879	298.5	4.079	72.68
36	A1, B1, C1, B2, H2	4.980	13.77	0.1812	8.66	0.115	1.88	0.7569	0.4072	268.4	3.769	71.21
37	A1, B1, C1, B1, H1	5.470	13.29	0.1792	8.67	0.111	0.86	0.8569	0.5522	337.8	4.687	72.06
38	A1, B1, C1, B1, H1	5.470	13.29	0.1792	8.67	0.111	1.02	0.8383	0.5283	329.3	4.586	71.79
39	A1, B1, C1, B1, H1	5.470	13.29	0.1792	8.67	0.111	1.02	0.7709	0.4841	310.3	4.217	73.58
40	A1, B1, C1, B1, H1	5.399	10.55	0.1790	8.68	0.094	2.19	0.7388	0.3155	283.3	3.909	71.02
41	A1, B1, C1, B1, H1	5.399	10.55	0.1790	8.68	0.094	2.19	0.7684	0.3662	293.7	4.149	70.79
42‡	A1, B1, C1, B1, H1	5.112	10.19	0.1801	8.69	0.092	1.22	0.8049	0.5159	293.7	4.115	71.37
43	A1, B1, C1, B1, H1	5.978	14.82	0.3565	8.71	0.146	1.72	0.8648	0.4780	369.7	5.170	71.51
44	A1, B1, C1, B1, H1	5.978	14.82	0.3565	8.71	0.146	1.72	0.8794	0.4809	374.7	5.257	71.28
45	A1, B1, C1, B1, H1	5.978	14.82	0.3565	8.71	0.146	1.72	0.8836	0.4986	356.9	4.983	71.62
46	A1, B1, C1, B1, H1	5.978	14.82	0.3565	8.71	0.146	1.72	0.7603	0.4593	331.2	4.545	72.87
47	A1, B1, C1, B2, H2	4.711	13.61	0.1985	9.01	0.099	1.91	0.7949	0.4851	260.3	3.745	69.50
48	A1, B1, C1, B2, H2	4.711	13.61	0.1985	9.01	0.099	1.91	0.7288	0.5937	244.6	3.433	71.26
49	A2, B3, C1, B2, H2	5.471	13.27	0.1797	9.01	0.095	1.34	0.6739	0.5013	272.0	3.887	73.78
50	A1, B3, C1, B2, H2	5.471	13.27	0.1797	9.01	0.095	1.34	0.6463	0.5588	251.4	3.536	71.09
51	A1, B1, C1, B2, H2	5.471	13.27	0.1797	9.01	0.095	1.31	0.6190	0.5196	246.2	3.387	72.71

‡ Solutions allowed to stand overnight.

‡Some $MgCl_2$ was present in these solutions in the following amounts: *Exper. No.* 32, 33, and 34, 0.120 mM; exper. no. 1, 22, 28, 34, and 42, 3.293 mM; *exper. no.* 28 and 30, 0.037 mM.

Table A3: Experimental data for the glucose/hexokinase reaction as a function of ionic strength. Temperature is 25.04°C.

Exper. No.	Materials	Glucose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of hexose reacted μmol	-Molar enthalpy, kJ·mol^{-1}
1	A2, E2, C1, B2, H2	1.1511	2.468	0.01911	8.66	0.0181	1.269	0.7202	0.4244	60.60	0.8291	73.09
2	A2, E2, C1, B2, H2	1.0531	2.530	0.01969	8.65	0.0186	0.899	0.7124	0.4930	51.29	0.7302	66.37
3	A2, E2, C1, B2, H2	1.0531	2.530	0.01959	8.65	0.0186	0.899	0.5869	0.5511	44.01	0.6181	71.20
4	A2, E2, C1, B2, H2	1.0531	2.530	0.01969	8.65	0.0186	0.899	0.6198	0.5042	44.10	0.6527	67.57
5	A2, E2, C1, B2, H2	2.3432	6.087	0.05427	8.71	0.0450	0.871	0.6212	0.5629	99.89	1.456	68.61
6	A2, E2, C1, B2, H2	2.3432	6.087	0.05427	8.71	0.0450	0.871	0.7162	0.5324	117.9	1.678	70.26
7	A2, E2, C1, B2, H2	2.3432	6.087	0.05427	8.71	0.0450	0.871	0.6553	0.5169	108.5	1.536	70.63
8	A1, E2, C1, B2, H2	2.6092	6.802	0.04752	8.67	0.0488	1.852	0.7676	0.4874	143.3	2.003	71.52
9	A2, E2, C1, B2, H2	5.0916	10.43	0.09134	8.68	0.0777	1.108	0.7812	0.5303	280.6	3.978	70.53
10	A2, E2, C1, B2, H2	5.0916	10.43	0.09134	8.68	0.0777	1.108	0.7768	0.5582	284.7	3.955	71.98
11	A2, E2, C1, B2, H2	4.9450	20.48	0.79490	8.72	0.249	0.872	0.7290	0.5431	264.9	3.605	73.47
12	A2, E2, C1, B2, H2	4.9450	20.48	0.79490	8.72	0.249	0.872	0.6811	0.5869	234.6	3.369	69.64
13	A2, E2, C1, B2, H2	4.9450	20.48	0.79490	8.72	0.249	0.872	0.6707	0.5175	231.3	3.316	69.75

Table A4 Experimental data for glucose/hexokinase reaction using the di-TRIS salt of ATP.

Exper. No.	Materials	Glucose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of hexose reacted μmol	-Molar enthalpy, kJ·mol^{-1}
1	A1, E1, C1, B1, H1	5.5980	17.34	0.1785	8.41	0.1533	0.931	0.8439	0.4496	331.5	4.724	70.17
2	A1, E1, C1, B1, H1	4.5502	9.962	0.1815	8.54	0.0996	1.77	0.7867	0.4465	257.7	3.580	71.99
3	A1, E1, C1, B1, H1	4.5502	9.962	0.1815	8.54	0.0996	1.77	0.7755	0.5064	252.3	3.529	71.50
4	A1, E1, C1, B1, H1	4.5502	9.962	0.1815	8.54	0.0996	1.77	0.8023	0.6742	355.0	3.651	69.84

Average = -70.87 kJ·mol^{-1}

Estimated standard deviation of the mean = 0.52 kJ·mol^{-1}

Table A' Experimental data for mannose/hexokinase reaction. Temperature is 25.04°C.

Exper. No.	Materials	Mannose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of hexose reacted μmol	-Molar enthalpy, kJ·mol^{-1}
1	A1, E1, C3, B1, H1	5.437	11.32	0.1789	8.57	0.1168	1.47	0.8858	0.5193	327.0	4.816	67.90
2	A1, E1, C2, B1, H1	5.437	11.32	0.1789	8.57	0.1168	1.47	0.8584	0.4655	325.1	4.667	69.66
3	A1, E1, C2, B1, H1	5.437	11.32	0.1789	8.57	0.1168	1.47	1.0499	0.4116	395.4	5.708	69.27
4	A1, E1, C2, B1, H1	5.029	10.42	0.1793	8.62	0.0963	1.80	0.8605	0.5378	306.4	4.327	70.35
5	A1, E1, C3, B1, H1	5.029	10.42	0.1793	8.62	0.0963	1.80	0.7801	0.4638	277.4	3.973	69.82
6	A1, E1, C3, B1, H1	5.029	10.42	0.1793	8.62	0.0963	1.80	0.9189	0.4839	318.5	4.621	68.92

Average = -69.32

Estimated standard deviation of the mean = 0.35 kJ·mol^{-1}

Table A' Experimental data for fructose/hexokinase reaction. Temperature is 25.04°C.

Exper. No.	Materials	Fructose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol TRIS·(kg solution)$^{-1}$	pH	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of hexose reacted μmol	-Molar enthalpy, kJ·mol^{-1}
1	A1, E1, C4, B1, H1	5.206	10.99	0.1786	8.61	0.1004	1.69	0.8435	0.4782	274.6	4.391	62.54
2	A1, E1, C4, B1, H1	5.206	10.99	0.1786	8.61	0.1004	1.69	0.8349	0.4906	277.5	4.446	63.85
3	A1, E1, C4, B1, H1	5.206	10.99	0.1786	8.61	0.1004	1.69	1.0100	0.4550	329.3	5.258	62.63
4	A1, E1, C5, B1, H1	6.156	10.88	0.1781	8.63	0.0984	0.91	0.8240	0.4647	314.4	5.073	61.98
5	A1, E1, C5, B1, H1	6.156	10.88	0.1781	8.63	0.0984	0.91	0.8177	0.5184	317.3	5.034	63.03
6	A1, E1, C5, B1, H1	6.156	10.88	0.1781	8.63	0.0984	0.91	0.8722	0.4717	325.8	5.169	60.68

Average = -62.45 kJ·mol^{-1}

Estimated standard deviation of the mean = 0.43 kJ·mol^{-1}

Table A7 Experiments performed at 26,51°C and 31.96°C.

Exper. No.	Materials	Hexose concentration, mmol·(kg solution)$^{-1}$	ATP concentration, mmol·(kg solution)$^{-1}$	Buffer concentration, mol·(kg solution)$^{-1}$	pH	Temperature (°C)	Ionic Strength	Enzyme concentration, g·(kg solution)$^{-1}$	Mass of substrate solution, g	Mass of enzyme solution, g	Measured heat, mJ	Quantity of hexose reacted, µmol	-Molar enthalpy, kJ·mol^{-1}
							Glucose						
1	A2, E2, C1, B2, H2	5.263	13.804	0.1834	8.63	26.51	0.117	1.294	0.7259	0.4999	276.1	3.820	72.28
2	A2, E2, C2, B2, H2	5.263	13.804	0.1834	8.63	26.51	0.117	1.294	0.5772	0.6199	218.7	3.038	71.99
3	A2, E2, C1, B2, H2	5.263	13.804	0.1834	8.63	26.51	0.117	1.294	0.6822	0.6547	258.9	3.590	72.12
												Average =	72.13
												Estimated standard deviation of the mean =	0.09
1	A2, E2, C1, B2, H2	5.915	14.079	0.1823	8.65	31.96	0.117	1.349	0.8835	0.5785	294.2	4.067	72.36
2	A2, E2, C1, B2, H2	5.915	14.079	0.1823	8.65	31.96	0.117	1.349	0.6151	0.5261	261.0	3.638	71.74
3	A2, E2, C1, B2, H2	5.915	14.079	0.1823	8.65	31.96	0.117	1.349	0.5983	0.4304	250.5	3.539	70.78
												Average =	71.62
												Estimated standard deviation of the mean =	0.45
							Mannose						
1	A2, E2, C3, B2, H2	5.536	14.252	0.1839	8.60	26.51	0.121	1.562	0.6986	0.5349	267.8	3.867	69.25
2	A2, E2, C3, B2, H2	5.536	14.252	0.1839	8.60	26.51	0.121	1.562	0.6490	0.5592	247.4	3.593	68.86
3	A2, E2, C3, B2, H2	5.536	14.252	0.1839	8.60	26.51	0.121	1.562	0.6426	0.5974	242.5	3.557	68.18
												Average =	68.76
												Estimated standard deviation of the mean =	0.31
1	A2, E2, C3, B2, H2	5.688	13.875	0.1813	8.63	31.96	0.117	2.171	0.7130	0.4739	280.8	4.056	69.23
2	A2, E2, C3, B2, H2	5.688	13.875	0.1813	8.63	31.96	0.117	2.171	0.6764	0.5838	266.5	3.847	69.27
3	A2, E2, C3, B2, H2	5.688	13.875	0.1813	8.63	31.96	0.117	2.171	0.5896	0.6214	230.6	3.354	68.75
												Average =	69.08
												Estimated standard deviation of the mean =	0.17
							Fructose						
1	A2, E2, C5, B2, H2	6.870	13.853	0.1806	8.63	26.51	0.116	1.600	0.6881	0.4444	296.5	4.729	62.72
2	A2, E2, C5, B2, H2	6.870	13.853	0.1806	8.63	26.51	0.116	1.600	0.7039	0.5708	304.1	4.850	62.70
3	A2, E2, C5, B2, H2	6.870	13.853	0.1806	8.63	26.51	0.116	1.600	0.6406	0.6006	276.2	4.401	63.44
												Average =	62.95
												Estimated standard deviation of the mean =	0.25
1	A2, E2, C5, B2, H2	6.277	14.279	0.1826	8.61	31.96	0.121	1.479	0.7249	0.5430	287.4	4.559	63.16
2	A2, E2, C5, B2, H2	6.277	14.279	0.1826	8.61	31.96	0.121	1.479	0.6569	0.5212	259.8	4.123	63.01
3	A2, E2, C5, B2, H2	6.277	14.279	0.1826	8.61	31.96	0.121	1.479	0.6673	0.5950	261.8	4.199	62.97
												Average =	63.05
												Estimated standard deviation of the mean =	0.06

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