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Thermodynamics of reactions catalyzed by L-iditol 2-dehydrogenase: the xylose assimilation pathway

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Apparent equilibrium constants and calorimetric enthalpies of reaction have been measured for the following enzyme catalyzed (L-iditol 2-dehydrogenase) biochemical reactions in phosphate buffer at pHs near 7.5 and at the temperature 298.15 K

$$\text{D-sorbitol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{D-fructose(aq)} + \text{NAD}_{red}\text{(aq)},$$

$$\text{L-iditol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{L-sorbose(aq)} + \text{NAD}_{red}\text{(aq)},$$

$$\text{xylitol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{D-xylulose(aq)} + \text{NAD}_{red}\text{(aq)}.$$

Here, NAD_{ox} is β-nicotinamide-adenine dinucleotide (oxidized form) and NAD_{red} is β-nicotinamide-adenine dinucleotide (reduced form). The results are used to calculate equilibrium constants and standard molar enthalpies, entropies, and Gibbs free energies for reference reactions involving specific species. Standard formation properties and standard transformed formation properties of the biochemical reactants are also calculated. The thermodynamics of the xylose assimilation pathway is summarized.

1. Introduction

The enzyme L-iditol 2-dehydrogenase (Enzyme Commission number 1.1.1.14),[1] also known as sorbitol dehydrogenase, catalyzes the following reactions in bacteria and mammals:†

$$\text{D-sorbitol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{D-fructose(aq)} + \text{NAD}_{red}\text{(aq)}, \quad (1)$$

$$\text{L-iditol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{L-sorbose(aq)} + \text{NAD}_{red}\text{(aq)}, \quad (2)$$

$$\text{xylitol(aq)} + \text{NAD}_{ox}\text{(aq)} = \text{D-xylulose(aq)} + \text{NAD}_{red}\text{(aq)}. \quad (3)$$

Reaction (3) is of importance to the conversion of hemicellulosic materials (a significant component of plants) to ethanol. In this application, the hemicellulose

† Abbreviations used in this paper follow. For species in chemical reference reactions: NAD^-, oxidized form of β-nicotinamide-adenine dinucleotide; $NADH^{2-}$, reduced form of β-nicotinamide-adenine dinucleotide; $NADP^{3-}$, oxidized form of β-nicotinamide-adenine dinucleotide phosphate; and $NADPH^{4-}$, reduced form of β-nicotinamide-adenine dinucleotide phosphate. For reactants in overall biochemical reactions: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; NAD_{ox}, β-nicotinamide-adenine dinucleotide (oxidized form); NAD_{red}, β-nicotinamide-adenine dinucleotide (reduced form); $NADP_{ox}$, β-nicotinamide-adenine dinucleotide phosphate (oxidized form); $NADP_{red}$, β-nicotinamide-adenine dinucleotide phosphate (reduced form). Tris is tris(hydroxymethyl)aminomethane.

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is first broken down into D-xylose, which then enters the xylose assimilation pathway.[2,3]

L-iditol 2-dehydrogenase also participates in the polyol pathway.[4] This is normally a minor metabolic pathway. However, in certain disease states, particularly diabetic hyperglycemia, this pathway assumes increased importance. Thus, intermediates such as D-sorbitol can accumulate in cells and cause a serious osmotic imbalance and consequent pathology in nerves and in tissues. One such example is the formation of cataracts.[5]

The principal aim of this study is to determine apparent equilibrium constants and calorimetric enthalpies for reactions (1), (2), and (3). These results are then used to calculate equilibrium constants and standard molar enthalpies for reference reactions that involve specific species. These quantities can be used to calculate the position of equilibrium of the corresponding biochemical reactions as a function of temperature, pH, and ionic strength. Standard formation properties and standard transformed formation properties[6] for these various substances are also obtained. These results are used in conjunction with results from the literature to gain insight into the operation of the xylose assimilation pathway.

2. Experimental

Pertinent information on the substances used in this study is given in table 1. Estimates of the purities of the D-fructose, L-iditol, D-sorbitol, L-sorbose, and xylitol were obtained chromatographically with a Dionex Bio-LC (see below). The use of this method for D-xylulose was not possible because D-xylulose appeared as a very broad peak on the chromatograms. However, the mole fraction purity of the D-xylulose was estimated by using L-iditol 2-dehydrogenase to catalyse the reaction of the D-xylulose with excess NAD_{red}. The chromatogram of this reaction mixture obtained with the Dionex BioLC showed that the reaction was complete (<0.005 mole fraction D-xylulose remained). The amount of xylitol formed in this reaction was also measured chromatographically and the result used to calculate a mole fraction purity of (0.919 ± 0.005) for the D-xylulose sample. This does not include the water in the sample, the mass fraction of which was determined by Karl Fischer titration. The supplier used g.c. to determine a mole fraction purity of ≈ 0.95 for this D-xylulose sample. The uncertainty given above for the D-xylulose mole fraction purity is based on three replicate measurements and is equal to two estimated standard deviations of the mean. Because the method used depends upon a knowledge of the purity of the xylitol, and is also subject to possible interferences, we believe that a more realistic estimate of the uncertainty is ± 0.03. This brings our result for the D-xylulose mole fraction purity into agreement with the approximate result of the analysis performed by g.c.

A Hewlett-Packard h.p.l.c. (see below) was used to obtain estimates of the mole fraction purities of the NAD_{ox} and NAD_{red}. In addition, the supplier used the following methods to characterize several of these substances: thin-layer chromatography for L-iditol and L-sorbose; enzymatic and spectrophotometric assays for the NAD_{ox} and NAD_{red}; and g.c. for the L-sorbose, D-sorbitol, and

xylitol. The estimates of the mole fraction purities obtained by the supplier with these methods were in agreement with the purities obtained in this laboratory with the aforementioned chromatographic techniques. The mass fractions of water in the substances (see table 1) were measured with a Metrohm Model 633 automatic Karl Fischer titrator. Information on the optical rotations α ($\lambda = 589.3$ nm, $T = 273.15$ K, mass concentration γ, path length = 10 cm) of these samples that was obtained from the vendors follows: D-fructose ($\gamma = 1\ \mathrm{g\cdot dm^{-3}}$), $\alpha = -92.4\cdot\pi/180$; L-iditol ($\gamma = 0.3\ \mathrm{g\cdot dm^{-3}}$), $\alpha = -3.3\cdot\pi/180$; L-sorbose ($\gamma = 1.2\ \mathrm{g\cdot dm^{-3}}$), $\alpha = -43.8\cdot\pi/180$; and D-xylulose ($\gamma = 0.1\ \mathrm{g\cdot dm^{-3}}$), $\alpha = -29\cdot\pi/180$.

Analyses for NAD_{ox} and NAD_{red} were done with a Hewlett-Packard h.p.l.c., a Serva DEAE Si 100 column thermostatted at $T = 311$ K, and a diode array detector set at a wavelength of 254 nm. Solutions I and II were used for the mobile phase: I, 0.02 $\mathrm{mol\cdot dm^{-3}}$ KH_2PO_4 at pH = 4.7; and II, 0.25 $\mathrm{mol\cdot dm^{-3}}$ K_2HPO_4 at pH = 7.47. The following linear gradient was used: solution I (volume fraction $\phi = 1.00$) at time $t = 0$; at $t = 15$ min the mobile phase consisted of solution I ($\phi = 0.50$) and

TABLE 1. Chemical Abstracts Services (CAS) registry numbers, empirical formulas, molar masses M, suppliers, mass fraction of water contents w determined by Karl Fischer analysis, and mole fraction purities x for the principal substances used in this study. The mole fraction purities do not include the water, or, for NAD_{red}, the ethanol in the sample

Substance	CAS registry number	Formula	M / $\mathrm{kg\cdot mol^{-1}}$	Supplier [a]	w [b,c]	x
dipotassium hydrogen phosphate	7758-11-4	K_2HPO_4	0.174176	S		0.995
D-fructose	57-48-7	$C_6H_{12}O_6$	0.180158	F	0.0026 ± 0.0008	>0.99
L-iditol	488-45-9	$C_6H_{14}O_6$	0.182174	S	0.0047 ± 0.0002	>0.99
L-iditol 2-dehydrogenase [d]	9028-21-1		≈ 41 [e]	S		
NAD_{ox}	53-84-9	$C_{21}H_{27}N_7O_{14}P_2$	0.663431	S	0.0659 ± 0.0005	0.99
NAD_{red}	606-68-8	$C_{21}H_{27}N_7O_{14}P_2Na_2$	0.709411	S	[f]	0.99
phosphoric acid	7664-38-2	H_3PO_4	0.97995	B		>0.999
D-sorbitol	50-70-4	$C_6H_{14}O_6$	0.182174	S	0.0058 ± 0.0009	0.98
L-sorbose	87-79-6	$C_6H_{12}O_6$	0.180158	S	0.0003 ± 0.0001	>0.99
xylitol	87-99-0	$C_5H_{12}O_5$	0.152147	S	0.0020 ± 0.0001	0.991
D-xylulose	551-84-8	$C_5H_{10}O_5$	0.150131	S	0.109 ± 0.005	0.919 [g]

[a] B = Baker, F = Fisher, and S = Sigma. Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

[b] All uncertainties given in this paper are, unless indicated otherwise, equal to two estimated standard deviations of the mean.

[c] The mass fraction moisture contents of dipotassium hydrogen phosphate and L-iditol 2-dehydrogenase were not measured. The phosphoric acid was a solution of known concentration.

[d] Lyophilized powder obtained from sheep liver.

[e] This approximate molar mass is based upon the structural information given by Eklund *et al.*[7]

[f] Two different samples of NAD_{red} were used. The mass fraction water contents were (0.0621 ± 0.0007) and (0.0616 ± 0.0008). These respective NAD_{red} samples were reported by the vendor to contain mass fractions of ethanol equal to 0.035 and 0.028.

[g] This mole fraction purity is based upon the measurement of the amount of xylitol formed by reaction of the D-xylulose sample with excess NAD_{red}.

solution II ($\phi = 0.50$). The flow rate was 0.0167 $cm^3 \cdot s^{-1}$. Typical retention times of NAD_{ox} and NAD_{red} were 9.3 min and 15.9 min, respectively.

A Dionex Bio-LC with a pulsed amperometric detector, a Dionex MA1 column, and a 1.0 $mol \cdot dm^{-3}$ NaOH mobile aqueous phase at a flow rate of 0.005 $cm^3 \cdot s^{-1}$ was used for the analyses of the sugars and sugar alcohols. Typical retention times were: 17.1 min and 23.0 min, respectively, for **D**-sorbitol and **D**-fructose; 15.7 min and 21.3 min, respectively, for **L**-iditol and **L**-sorbose; and 14.4 min and ≈ 29 min, respectively, for xylitol and **D**-xylulose. The peaks corresponding to **D**-xylulose were very broad and could not be integrated reliably.

A Beckman DU-70 spectrophotometer was used to measure the changes in the (decadic) absorbances A of the reaction mixtures. At the temperature $T = 298.15$ K and a wavelength $\lambda = 340$ nm, the molar (decadic) absorption coefficient ε of NAD_{red} is $6.292 \cdot 10^4$ $dm^2 \cdot mol^{-1}$, while ε of NAD_{ox} is < 25 $dm^2 \cdot mol^{-1}$.[8] The remaining reactants, *i.e.* the sugars and sugar alcohols, do not absorb at $\lambda = 340$ nm. Thus, any change in the absorbance of a reaction mixture is attributed to a change in the concentration of NAD_{red}. The cuvettes placed in this spectrophotometer had a nominal pathlength of 1.00 cm and were thermostatted at $T = 298.15$ K. The accuracy of the thermostat was confirmed by measuring the temperature inside a cuvette in the spectrophotometer with a thermistor that, in turn, had been calibrated with a National Institute of Standards and Technology (NIST) calibrated standard platinum resistance thermometer. The spectrophotometer was also calibrated with NIST standard reference materials no. 2031 (calibrated glass filters for absorbance) and no. 2034 (holmium oxide solution for λ).

The pHs of the reaction mixtures were measured with a combination glass micro-electrode and an Orion Model 811 pH meter. All pH measurements were made at the temperature at which the reactions occurred. Calibration of the pH meter was performed with a standard buffer prepared from KH_2PO_4 ($m = 0.009695$ $mol \cdot kg^{-1}$) and Na_2HPO_4 ($m = 0.03043$ $mol \cdot kg^{-1}$). These phosphates are standard reference materials 186-Id and 186-IId, respectively, from the National Institute of Standards and Technology. Intercomparisons of this "physiological" buffer against Fisher buffers certified at pH = 7.00, 8.00, and 9.00 were also done with agreement to within ± 0.03.

The method used for the equilibrium measurements is now described. First, a series of solutions was prepared in Teflon stoppered glass bottles. These solutions contained all four reactants (**D**-sorbitol, NAD_{red}, **D**-fructose, and NAD_{ox}) at molalities that resulted in apparent reaction quotients Q' close to the estimated value of the apparent equilibrium constant K' under the conditions of measurement. The initial absorbances ($\lambda = 340$ nm, $T = 298.15$ K) of these reaction mixtures were then measured. Following addition of a solution containing the enzyme (**L**-iditol 2-dehydrogenase), the absorbances were again measured. A suitable period of time for reaction was allowed, and the reaction mixtures were judged to be at equilibrium when the changes in the absorbances with time were comparable with that observed for the change in the absorbance of an (NAD_{red} + buffer) solution at the same T and pH. On this basis, the reaction(s) reached equilibrium within ≈ 2 h.

All solutions were prepared gravimetrically. Appropriate corrections were applied for loss of solution following the initial determinations of the absorbances of the reaction mixtures and the return of these solutions from the cuvettes to the bottles in which the solutions had been prepared and to which the enzyme was subsequently added. Also, the (numbered) cuvettes were rinsed with distilled water and air-dried following the initial determination of the absorbances of the reaction mixtures. Appropriate corrections, based upon separate control experiments, were applied for the absorbance of the enzyme solution and for the observed change in absorbance of the NAD_{red} with time (NAD_{red} drift). The correction to the absorbances of the reaction mixtures due to the absorption of the enzyme was ≈ -0.0043. The correction for the NAD_{red} drift was $2.5 \cdot 10^{-6} \cdot A \cdot s^{-1}$. Since these experiments lasted $\approx 7.2 \cdot 10^3$ s, the drift corrections were $\approx 0.02 \cdot A$.

The apparent reaction quotients Q' were selected so that Q' (min) $< K' < Q'$ (max), where K' is the presumed value of the apparent equilibrium constant and Q' (min) and Q' (max) are, respectively, the minimum and maximum values of the apparent equilibrium quotient used in the experiments. The experimental results were then used to construct a plot of Q' as a function of the change in absorbance ΔA. In such a plot, or in a numerical fit of Q' against ΔA, the value of Q' corresponding to $\Delta A = 0$ is K'. This method presupposes a knowledge of an approximate value of K', and it will not work if the presumed value of K' is not in the aforementioned range. In this study, approximate values of K' were obtained from results in the literature as well as from preliminary experiments in which equilibrium was approached from both extreme ends of the reaction. However, even if the condition Q' (min) $< K' < Q'$ (max) is not met, the results of an unsuccessful experiment will lead to a better estimate of K' and, very likely, to a successful next experiment.

The reaction mixtures having the smallest absolute value of ΔA for a given reaction were analysed with the h.p.l.c. methods described above. Here, solutions for the determination of the response factors were prepared from the same stock solutions of reagents used for the preparation of the reaction mixtures in the spectrophotometric measurements. Values of the apparent equilibrium constants K' were calculated from the chromatographically determined molalities. However, the molalities of NAD_{ox} and **D**-xylulose in reaction (3) could not be measured with the h.p.l.c. for the following reasons. The detector was saturated at the molality of the NAD_{ox} used in this set of experiments and the **D**-xylulose chromatographic peak was very broad and could not be integrated reliably. Thus, for reaction (3), the initial molalities of NAD_{ox} and **D**-xylulose and the extent of reaction, as determined from the measured changes in the molalities of NAD_{red} and xylitol, were used to calculate K' from the h.p.l.c. results.

Three heat-conduction microcalorimeters were used for enthalpy of reaction measurements. They were calibrated electrically with a high stability d.c. power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results are

given in references 9 and 10. The sample vessels, fabricated from high-density polyethylene, contained two compartments that held $\approx 0.55\ cm^3$ and $\approx 0.45\ cm^3$ of solution. A stock solution III consisting of phosphate buffer and NAD_{red} was made before the preparation of the other solutions used in the calorimetric experiments. The substrate solution (placed in the $0.55\ cm^3$ compartment) consisted of a sugar (**D**-fructose, **L**-sorbose, or **D**-xylulose) dissolved in solution III. The enzyme solution (placed in the $0.45\ cm^3$ compartment) consisted of **L**-iditol 2-dehydrogenase dissolved in a separate portion of solution III. The molality of the NAD_{red} in these solutions was in excess of the amount required for complete reaction of the respective sugar.

The vessels and their contents were allowed to equilibrate in the calorimeters for 1 h before the enzyme and substrate solutions were mixed. Following reaction in the calorimeter for 60 min to 90 min, the reaction vessels were removed from the calorimeters and their contents were promptly analysed with the Dionex h.p.l.c. Thus, it was found that the mole fractions of unreacted **D**-fructose, **L**-sorbose, and **D**-xylulose were, respectively, 0.147, 0.200, and <0.005. Appropriate corrections were applied for incomplete reaction. The "blank" enthalpy changes for the mixing of the substrate solutions with solution III ranged from 0.29 mJ to 0.96 mJ; for the mixing of the enzyme solutions with solution III they ranged from -0.13 mJ to -0.60 mJ. These "blank" enthalpies of mixing were also applied as corrections to the measured enthalpy changes which were approximately -125 mJ, -78 mJ, and -304 mJ for the respective reactions involving **D**-fructose, **L**-sorbose, and **D**-xylulose. There were changes in the pHs of the reaction mixtures as a consequence of the reactions. Thus, for the reactions involving **D**-fructose, **L**-sorbose, and **D**-xylulose, {pH(final) - pH(initial)} was 0.17, 0.13, and 0.16, respectively. The positive signs indicate that protons were absorbed as a consequence of the reaction.

3. Results and discussion

The apparent equilibrium constants corresponding to the overall biochemical reactions (1), (2), and (3) are, respectively

$$K' = m(\textbf{D}\text{-fructose})\cdot m(NAD_{red})/\{m(\textbf{D}\text{-sorbitol})\cdot m(NAD_{ox})\}, \quad (4)$$

$$K' = m(\textbf{L}\text{-sorbose})\cdot m(NAD_{red})/\{m(\textbf{L}\text{-iditol})\cdot m(NAD_{ox})\}, \quad (5)$$

$$K' = m(\textbf{D}\text{-xylulose})\cdot m(NAD_{red})/\{m(\text{xylitol})\cdot m(NAD_{ox})\}. \quad (6)$$

The molalities m in equations (4), (5), and (6) are the total molalities of the various charged and uncharged species that are formed from the dissociation of the various substances in solution. As **D**-fructose, **L**-sorbose, **D**-xylulose, **D**-sorbitol, **L**-iditol, and xylitol do not ionize except in extremely alkaline solutions, the only dissociations of concern are those involving NAD_{ox} and NAD_{red}. These dissociations are dealt with below.

In discussing the thermodynamics of these reactions, it is useful to introduce the reference reactions:

$$\text{D-sorbitol(aq)} + \text{NAD}^-\text{(aq)} = \text{D-fructose(aq)} + \text{NADH}^{2-}\text{(aq)} + \text{H}^+\text{(aq)}, \quad (7)$$

$$\text{L-iditol(aq)} + \text{NAD}^-\text{(aq)} = \text{L-sorbose(aq)} + \text{NADH}^{2-}\text{(aq)} + \text{H}^+\text{(aq)}, \quad (8)$$

$$\text{xylitol(aq)} + \text{NAD}^-\text{(aq)} = \text{D-xylulose(aq)} + \text{NADH}^{2-}\text{(aq)} + \text{H}^+\text{(aq)}. \quad (9)$$

The equilibrium constants for reactions (7), (8), and (9) are

$$K_m = m(\text{D-fructose}) \cdot m(\text{NADH}^{2-}) \cdot m(\text{H}^+) / \{m(\text{D-sorbitol}) \cdot m(\text{NAD}^-) \cdot m^\circ\}, \quad (10)$$

$$K_m = m(\text{L-sorbose}) \cdot m(\text{NADH}^{2-}) \cdot m(\text{H}^+) / \{m(\text{L-iditol}) \cdot m(\text{NAD}^-) \cdot m^\circ\}, \quad (11)$$

$$K_m = m(\text{D-xylulose}) \cdot m(\text{NADH}^{2-}) \cdot m(\text{H}^+) / \{m(\text{xylitol}) \cdot m(\text{NAD}^-) \cdot m^\circ\}. \quad (12)$$

Here the molalities refer to specific species. Note that all species without charge numbers in reactions (7) to (9) and equations (10) to (12) are neutral. Since reactions (1), (2), and (3) are symmetric, the values of the corresponding apparent equilibrium constants are independent of the choice of standard state. However, reactions (7), (8), and (9) are not symmetric and the values of the corresponding equilibrium constants depend on the choice of standard state. The standard state of the solute used herein is the hypothetical ideal solution of unit molality ($m^\circ = 1$ mol·kg^{-1}); the standard pressure p° is 0.1 MPa. The quantity m° has been placed in the denominators of equations (10), (11), and (12) to make the equilibrium constants dimensionless.

The results obtained with the various reaction mixtures and which correspond to reactions (1), (2), and (3) are given in table 2. Polynomial fits of Q' as a function of the change in absorbance ΔA with terms to $(\Delta A)^3$ were found to represent the results adequately. The value of Q' which corresponds to $\Delta A = 0$ is equal to the apparent equilibrium constant K'. Thus, the first term in the polynomial fit for each set of results and its standard deviation yielded the following results: $K' = (0.092 \pm 0.003)$, $K' = (0.180 \pm 0.005)$, and $K' = (1.22 \pm 0.02) \cdot 10^{-3}$ for reactions (1), (2), and (3), respectively. Here the uncertainties are equal to two standard deviations. However, we believe that these uncertainties should be increased because the correction for the absorbance of the enzyme and for the "NAD_{red} drift" (see Experimental section) could be uncertain by as much as 20 per cent. This leads to an additional uncertainty of ± 0.001 in K' for reactions (1) and (2), and ± 0.02 in K' for reaction (3). Thus, the results obtained with the spectrophotometric method are: $K' = (0.092 \pm 0.004)$, $K' = (0.180 \pm 0.006)$, and $K' = (1.22 \pm 0.04) \cdot 10^{-3}$ for reactions (1), (2), and (3), respectively.

The following results were obtained from the h.p.l.c. measurements: $K' = (0.102 \pm 0.009)$, $K' = (0.199 \pm 0.009)$, and $K' = (1.21 \pm 0.05) \cdot 10^{-3}$ for reactions (1), (2), and (3), respectively. These two standard deviation uncertainties were obtained from the standard deviations of the molalities of the reactants as determined by h.p.l.c. and of the response factors used to calculate these molalities. It is seen that the spectrophotometric and h.p.l.c. results for reactions (1) and (3) are in

TABLE 2. Molalities of substances m in reaction mixtures, pHs, apparent reaction quotients Q', and measured changes in absorbance ΔA. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. The changes in absorbance have been corrected for the absorbance of added enzyme (**L**-iditol 2-dehydrogenase) and for drift in the absorbance of NAD_{red}. The mass fraction of the lyophilized enzyme in these solutions was ≈ 0.0013. The calculated ionic strengths I_m for the reaction mixtures for biochemical reactions (1), (2), and (3) are, respectively, 0.197 mol·kg^{-1}, 0.191 mol·kg^{-1}, and 0.189 mol·kg^{-1}. Uncertainties are discussed in the text

Biochemical reaction (1): $NAD_{ox}(aq)$ + **D**-sorbitol(aq) = $NAD_{red}(aq)$ + **D**-fructose(aq)

pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	$10^4 \cdot m(NAD_{ox})$ / mol·kg^{-1}	$10^4 \cdot m$(**D**-sorbitol) / mol·kg^{-1}	$10^4 \cdot m(NAD_{red})$ / mol·kg^{-1}	$10^4 \cdot m$(**D**-fructose) / mol·kg^{-1}	Q'	ΔA
7.63	0.09860	0.01047	4.271	8.314	1.706	4.362	0.2096	−0.3787
7.64	0.09861	0.01047	5.382	7.837	1.631	4.256	0.1646	−0.2840
7.63	0.09861	0.01047	6.268	7.515	1.582	4.121	0.1384	−0.1998
7.63	0.09862	0.01048	7.185	7.133	1.557	3.902	0.1185	−0.1348
7.62	0.09863	0.01048	9.140	6.582	1.400	3.632	0.08453	0.0444
7.61	0.09864	0.01048	10.55	6.083	1.316	3.409	0.06987	0.1371

Biochemical reaction (2): NAD_{ox} (aq) + **L**-iditol (aq) = NAD_{red} (aq) + **L**-sorbose (aq)

pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	$10^4 \cdot m(NAD_{ox})$ / mol·kg^{-1}	$10^4 \cdot m$(**L**-iditol) / mol·kg^{-1}	$10^4 \cdot m(NAD_{red})$ / mol·kg^{-1}	$10^4 \cdot m$(**L**-sorbose) / mol·kg^{-1}	Q'	ΔA
7.58	0.09591	0.01115	3.575	9.180	1.726	8.440	0.4440	−0.4665
7.58	0.09592	0.01115	4.912	8.917	1.639	7.994	0.2992	−0.2820
7.57	0.09592	0.01115	6.422	8.403	1.513	7.719	0.2164	−0.1017
7.57	0.09593	0.01115	7.437	7.873	1.517	7.305	0.1892	−0.0235
7.58	0.09594	0.01116	10.45	6.992	1.333	6.408	0.1169	0.2554
7.57	0.09595	0.01116	12.48	6.468	1.212	5.618	0.08432	0.4280

Biochemical reaction (3): xylitol(aq) + NAD_{ox} (aq) = **D**-xylulose(aq) + NAD_{red}(aq)

pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	$10^3 \cdot m(NAD_{ox})$ / mol·kg^{-1}	$10^3 \cdot m$(xylitol) / mol·kg^{-1}	$10^4 \cdot m(NAD_{red})$ / mol·kg^{-1}	$10^5 \cdot m$**D**-xylulose) / mol·kg^{-1}	$10^3 \cdot Q'$	ΔA
7.56	0.09526	0.01107	1.252	3.374	1.722	8.898	3.628	−0.2039
7.53	0.09533	0.01108	2.492	3.006	1.576	8.006	1.684	−0.0683
7.51	0.09536	0.01108	3.024	2.860	1.493	7.614	1.315	−0.0198
7.50	0.09540	0.01108	3.727	2.687	1.407	6.898	0.969	0.0802
7.48	0.09545	0.01109	4.524	2.392	1.263	6.487	0.757	0.1980

agreement, but that the spectrophotometric and h.p.l.c. results for reaction (2) differ by an amount (0.004) that is slightly outside the stated uncertainties. In any case, we calculate a weighted average of the spectrophotometric and h.p.l.c. results: $K' = (0.094 \pm 0.004)$, $K' = (0.186 \pm 0.005)$, and $K' = (1.22 \pm 0.03)\cdot 10^{-3}$ for reactions (1), (2), and (3), respectively. However, we judge that impurities in the biochemicals used in this study could cause errors as large as $0.03 \cdot K'$ for reactions (1) and (2) and $0.05 \cdot K'$ for reaction (3). On this basis, we increase the uncertainties and arrive at the final set of results: $K' = (0.094 \pm 0.005)$, $K' = (0.186 \pm 0.008)$, and $K' = (1.22 \pm 0.07)\cdot 10^{-3}$ for reactions (1), (2), and (3), respectively.

Results for the calorimetrically determined molar enthalpies $\Delta_r H_m$(cal) for the reverse of reactions (1), (2), and (3) are given in table 3. We believe that the statistical uncertainties for reactions (1) and (2) given in this table are sufficiently large to encompass possible systematic errors in the measurements. However, we judge that a reasonable uncertainty in $\Delta_r H_m$(cal) due to the uncertainty in the

TABLE 3. Results of calorimetric measurements for the reverse of biochemical reactions (1), (2), and (3) in phosphate buffer at $T = 298.15$ K. The molalities m 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 ionic strengths I_m are calculated. The mass fraction of the L-iditol dehydrogenase in the final solutions was ≈ 0.0004. The uncertainties are equal to two estimated standard deviations of the mean

Reverse of biochemical reaction (1):
D-fructose(aq) + NAD_{red}(aq) = D-sorbitol(aq) + NAD_{ox}(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	m(D-fructose) / mol·kg^{-1}	$m(NAD_{red})$ / mol·kg^{-1}	I_m / mol·kg^{-1}	$\Delta_r H_m$(cal) / kJ·mol^{-1}
1	7.55	0.0994	0.0167	0.00893	0.0132	0.217	−17.32
2	7.55	0.0994	0.0167	0.00872	0.0132	0.217	−17.19
3	7.55	0.0994	0.0167	0.00856	0.0132	0.217	−17.35
4	7.55	0.0994	0.0167	0.00908	0.0132	0.217	−16.77
5	7.55	0.0994	0.0167	0.00865	0.0132	0.217	−16.77
$\langle \Delta_r H_m(\text{cal}) \rangle = -(17.1 \pm 0.3)$ kJ·mol^{-1}							

Reverse of biochemical reaction (2):
L-sorbose(aq) + NAD_{red}(aq) = L-iditol(aq) + NAD_{ox}(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	m(L-sorbose) / mol·kg^{-1}	$m(NAD_{red})$ / mol·kg^{-1}	I_m / mol·kg^{-1}	$\Delta_r H_m$(cal) / kJ·mol^{-1}
1	7.39	0.102	0.0191	0.00945	0.0125	0.215	−10.20
2	7.39	0.102	0.0191	0.00955	0.0125	0.215	−9.67
3	7.39	0.102	0.0191	0.00929	0.0125	0.215	−11.52
4	7.39	0.102	0.0191	0.00886	0.0125	0.215	−10.21
5	7.39	0.102	0.0191	0.00899	0.0125	0.215	−10.79
6	7.39	0.102	0.0191	0.00885	0.0125	0.215	−10.42
$\langle \Delta_r H_m(\text{cal}) \rangle = -(10.5 \pm 0.5)$ kJ·mol^{-1}							

Reverse of biochemical reaction (3):
D-xylulose(aq) + NAD_{red}(aq) = xylitol(aq) + NAD_{ox}(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	m(D-xylulose) / mol·kg^{-1}	$m(NAD_{red})$ / mol·kg^{-1}	I_m / mol·kg^{-1}	$\Delta_r H_m$(cal) / kJ·mol^{-1}
1	7.43	0.102	0.0191	0.00868	0.0127	0.218	−35.12
2	7.43	0.102	0.0191	0.00917	0.0127	0.218	−34.89
3	7.43	0.102	0.0191	0.00866	0.0127	0.218	−35.63
4	7.43	0.102	0.0191	0.00881	0.0127	0.218	−35.06
5	7.43	0.102	0.0191	0.00910	0.0127	0.218	−35.12
6	7.43	0.102	0.0191	0.00918	0.0127	0.218	−35.32
$\langle \Delta_r H_m(\text{cal}) \rangle = -(35.2 \pm 0.2)$ kJ·mol^{-1}							

purity of the D-xylulose sample is $\approx 0.03 \cdot \Delta_r H_m(\text{cal}) \approx 1.1$ kJ·mol^{-1}. Thus, for the reaction conditions given in table 3, $\Delta_r H_m(\text{cal}) = (17.1 \pm 0.3)$ kJ·mol^{-1}, $\Delta_r H_m(\text{cal}) = (10.5 \pm 0.5)$ kJ·mol^{-1}, and $\Delta_r H_m(\text{cal}) = (35.2 \pm 1.1)$ kJ·mol^{-1} for reactions (1), (2), and (3), respectively.

Information on the dissociation constants of the reactants is needed to relate the results for the reactions (1), (2), and (3), expressed in terms of apparent

equilibrium constants involving sums of molalities of species, to the reference reactions (7), (8), and (9) that involve specific species. Moore and Underwood[11] performed a potentiometric titration of NAD_{ox} and obtained pK = (3.88 ± 0.02) for the dissociation of NAD^0(aq) to NAD^-(aq) at $T = 298.15$ K and ionic strength $I = 0$. We estimate pK ≈ 3.9 for the dissociation of $NADH^-$(aq) to $NADH^{2-}$(aq) at $T = 298.15$ K and $I = 0$ on the basis of the structural similarity of NAD_{ox} and NAD_{red}. At the pHs used in this study, the predominant species (mole fraction $x > 0.999$) of NAD_{ox} and NAD_{red} have charge numbers of −1 and −2, respectively. Since D-fructose, L-sorbose, D-xylulose, D-sorbitol, L-iditol, and xylitol do not ionize except in extremely alkaline solutions, the predominant forms of these substances are the neutral species. In summary, the predominant species at the pHs used in this study are those shown in the reference reactions (7), (8), and (9). On the basis of stoichiometry, the change in binding of the hydrogen ion $\Delta_r N(H^+) = -1.00$ for reactions (1), (2), and (3) at the pHs used in this study. Thermodynamic quantities for the dissociation of the phosphate buffer are also needed for the equilibrium calculations to follow. We have used $K_m = 6.23 \cdot 10^{-8}$ and $\Delta_r H^\circ_m = 4.2$ kJ·mol^{-1} at $T = 298.15$ K and $I = 0$ for the dissociation of $H_2PO_4^-$(aq):

$$H_2PO_4^-(aq) = HPO_4^{2-}(aq) + H^+(aq). \quad (13)$$

These values were calculated from the standard formation properties given in reference 12.

The results given in tables 2 and 3 can be used together with the above thermodynamic quantities for the dissociation constants and standard molar enthalpies of dissociation and a previously described[13] equilibrium model to obtain thermodynamic quantities for the reference reactions (7), (8), and (9). This calculation is particularly simple since there is one predominant reaction in each case and, at a fixed ionic strength, $K_m(\text{ref}) = K'_m \cdot m(H^+)$. However, it is still necessary to make adjustments to various quantities for ionic strength. To do this, we have used an estimated "ion-size" parameter of 1.6 kg$^{1/2}$·mol$^{-1/2}$ in the extended Debye-Hückel equation to estimate the activity coefficients of the aqueous species in solution.

From these equilibrium calculations, we obtain $K_m = (9.0 \pm 0.8) \cdot 10^{-10}$, $K_m = (20.2 \pm 1.8) \cdot 10^{-10}$, and $K_m = (0.153 \pm 0.015) \cdot 10^{-10}$ for the reference reactions (7), (8), and (9), respectively, at $T = 298.15$ K and $I = 0$. Similarly, from the results given in table 3, we calculate $\Delta_r H^\circ_m = (21.3 \pm 0.3)$ kJ·mol^{-1}, $\Delta_r H^\circ_m = (14.7 \pm 0.5)$ kJ·mol^{-1}, and $\Delta_r H^\circ_m = (39.4 \pm 1.1)$ kJ·mol^{-1} for the reference reactions (7), (8), and (9), respectively, at $T = 298.15$ K and $I = 0$. The uncertainties in these thermodynamic quantities have two components: possible errors in the measurement of the apparent equilibrium constants and calorimetric enthalpies of reaction, and possible errors in the quantities used in the equilibrium model. The uncertainty in the activity coefficient model is the major factor in the equilibrium model component. The effects of these possible errors on the calculated thermodynamic quantities were determined by perturbing both the measured apparent equilibrium constants and calorimetric enthalpies of reaction and the

"ion-size" parameter used in the activity coefficient model by the final uncertainties given in the discussion above. An uncertainty of $\pm 0.3\ \mathrm{kg^{1/2}\cdot mol^{-1/2}}$ was assumed for the "ion-size" parameter in the activity coefficient model. The effects due to these perturbations were then combined in quadrature to obtain the estimated uncertainties assigned to the thermodynamic quantities for the reference reactions (7), (8), and (9). It was found that the uncertainties in the equilibrium constants for reference reactions (7), (8), and (9) due to perturbations of the "ion-size" parameter by the aforementioned amount were ≈ 50 per cent larger than the components in the uncertainties obtained from the perturbation where only the uncertainties in the measured apparent equilibrium constants were used. Since reactions (7), (8), and (9) are charge asymmetric, this is not unexpected. However, perturbation of the "ion-size" parameter caused no change in the calculated standard molar enthalpies for reactions (7), (8), and (9). The reason is that when, for example, the reverse of reaction (7) is carried out in phosphate buffer, the change in binding of $H^+(aq)$, $\Delta_r N(H^+)$, is equal to 1.00, and this is compensated by the release of one $H^+(aq)$ from the phosphate buffer. Thus, the net reaction is the sum of reaction (13) and the reverse of reaction (7):

$$\text{D-fructose(aq)} + \mathrm{NADH^{2-}(aq)} + \mathrm{H_2PO_4^-(aq)} = \text{D-sorbitol(aq)} + \mathrm{NAD^-(aq)} + \mathrm{HPO_4^{2-}(aq)}. \quad (14)$$

Since this reaction is charge symmetric, $\{\Delta_r H_m^\circ(I) - \Delta_r H_m^\circ(I=0)\} = 0$ within the assumption of the Debye-Hückel model. The equilibrium constants and standard molar enthalpies for the reference reactions were used to calculate the values of the standard molar Gibbs free energies and standard molar entropies given in table 4.

Thermodynamic results from the literature are summarized in table 5. For purposes of comparison, convenient quantities are the equilibrium constants K_m for the reference reactions (7), (8), and (9). The result $K_m = (9.0 \pm 0.8)\cdot 10^{-10}$ ($T = 298.15$ K and $I = 0$) obtained in the present study for reference reaction (7) is in good agreement with the value $9.5\cdot 10^{-10}$ obtained by Chakravorty *et al.*[17] The results obtained from the other studies[14–16,18,19] for reference reaction (7) range

TABLE 4. Equilibrium constants K_m, standard molar enthalpies $\Delta_r H_m^\circ$, standard molar Gibbs free energies $\Delta_r G_m^\circ$, and standard molar entropies $\Delta_r S_m^\circ$ for reactions (7), (8), and (9) at $T = 298.15$ K and $I = 0$. The basis of the uncertainties is discussed in the text

Reaction	$10^{10}\cdot K_m$	$\frac{\Delta_r H_m^\circ}{\mathrm{kJ\cdot mol^{-1}}}$	$\frac{\Delta_r G_m^\circ}{\mathrm{kJ\cdot mol^{-1}}}$	$\frac{\Delta_r S_m^\circ}{\mathrm{J\cdot K^{-1}\cdot mol^{-1}}}$
D-sorbitol(aq) + NAD^-(aq) = D-fructose(aq) + $NADH^{2-}$(aq) + H^+(aq) [a]	9.0 ± 0.8	21.3 ± 0.3	51.63 ± 0.23	-101.7 ± 0.4
L-iditol(aq) + NAD^-(aq) = L-sorbose(aq) + $NADH^{2-}$(aq) + H^+(aq) [b]	20.2 ± 1.8	14.7 ± 0.5	49.63 ± 0.23	-117.2 ± 0.6
xylitol(aq) + NAD^-(aq) = D-xylulose(aq) + $NADH^{2-}$(aq) + H^+(aq) [c]	0.153 ± 0.015	39.4 ± 1.1	61.73 ± 0.26	-74.9 ± 1.2

[a] Reaction (7). [b] Reaction (8). [c] Reaction (9).

TABLE 5. Results from the literature for biochemical reactions (1), (2), and (3). The thermodynamic temperatures T, the pH, the buffers and their concentrations, the $MgCl_2$ concentrations $c(MgCl_2)$, and the apparent equilibrium constants K' are given for each study. Approximate (concentration) ionic strengths I_c were calculated from the information given on the compositions of the solutions used in each study. We have calculated the values of the equilibrium constants K_m (column 7) for the respective reference reactions (7), (8), and (9) at $T = 298.15$ K and $I = 0$

Biochemical reaction (1): **D**-sorbitol(aq) + NAD_{ox}(aq) = **D**-fructose(aq) + NAD_{red}(aq)							
$\frac{T}{K}$	pH	Buffer	$\frac{c(MgCl_2)}{mol\cdot dm^{-3}}$	$\frac{I_c}{mol\cdot dm^{-3}}$	K'	$10^{10}\cdot K_m$	Reference
293.15	8.0	phosphate (0.00432 mol·dm^{-3})	0	0.012	0.24 [a]	20 [b]	Blakley[14]
298.15	≈7.0 [c]	?	?	?	0.021 [a]	≈20	Williams-Ashman and Banks[15]
298.15	7.87 [d]	Tris (0.05 mol·dm^{-3})	0.008	0.057	0.031 [a]	2.3	Hollmann[16]
296.15	8.6	glycine (0.080 mol·dm^{-3}) + KOH	0	0.006	0.454 [a]	9.5 [b]	Chakravorty *et al.*[17]
298.15	7.0	phosphate (0.1 mol·dm^{-3})	0	0.22	0.0058	2.3	Schneider and Giffhorn[18]
298.15	7.0	phosphate (0.1 mol·dm^{-3})	0	0.22	0.032	13	Schneider and Giffhorn[19]
Biochemical reaction (2): **L**-iditol(aq) + NAD_{ox}(aq) **L**-sorbose(aq) + NAD_{red}(aq)							
$\frac{T}{K}$	pH	Buffer	$\frac{c(MgCl_2)}{mol\cdot dm^{-3}}$	$\frac{I_c}{mol\cdot dm^{-3}}$	K'	$10^{10}\cdot K_m$	Reference
298.15	7.87 [d]	Tris (0.05 mol·dm^{-3})	0.008	0.057	0.031 [a]	2.3	Hollmann[16]
Biochemical reaction (3): xylitol(aq) + NAD_{ox}(aq) **D**-xylulose(aq) + NAD_{red}(aq)							
$\frac{T}{K}$	pH	Buffer	$\frac{c(MgCl_2)}{mol\cdot dm^{-3}}$	$\frac{I_c}{mol\cdot dm^{-3}}$	K'	$10^{10}\cdot K_m$	Reference
298.15	7.87 [d]	Tris (0.05 mol·dm^{-3})	0.008	0.057	0.0031 [a]	0.23	Hollmann[16]
296.15	8.6	glycine (0.080 mol·dm^{-3}) + KOH	0	0.006	0.0215 [a]	0.47 [b]	Chakravorty *et al.*[17]
298.15	9.5	glycine (0.030 mol·dm^{-3}) + NaOH	0	0.011	0.060	0.14	Sugai and Veiga[20]
298.15	7.0	Tris (0.05 mol·dm^{-3})	0.005	0.071	≈$4\cdot10^{-4}$	≈0.2	Ditzelmüller *et al.*[21]
298.15	7.0	Tris (0.05 mol·dm^{-3})	0.050	0.20	$6.9\cdot10^{-4}$	0.28	Rizzi *et al.*[22]

[a] Calculated from reported value of $K'\cdot10^{-pH}$.
[b] Adjusted to $T = 298.15$ K with the appropriate standard molar enthalpy of reaction determined in this study.
[c] Williams-Ashman and Banks[15] did not report the composition of the solution used in their measurements. The pH was assumed to be near 7.0.
[d] This is the average pH at which measurements were performed.

from $2.3\cdot10^{-10}$ to $20\cdot10^{-10}$ and have a mean value of $11\cdot10^{-10}$, not far from the result obtained in this study. Our result $K_m = (20.2 \pm 1.8)\cdot10^{-10}$ for reference reaction (8) differs by a factor of 10 from the result $2.3\cdot10^{-10}$ obtained from the study of Hollmann.[16] For reference reaction (9), the values of K_m vary over a more narrow range ($1.4\cdot10^{-11}$ to $4.7\cdot10^{-11}$); the mean is $2.7\cdot10^{-11}$. Our result, $K_m = (1.53 \pm 0.5)\cdot10^{-11}$, falls in the lower end of this range. There are several factors that lend confidence to the results obtained in the present study: two different methods of measurement (spectrophotometry and h.p.l.c.) were used; the materials were well characterized; the biochemicals were present in solution for only a few hours, thus minimizing the possibility of side reactions; and the position of equilibrium was approached from two different directions. Of the studies cited in table 5, equilibrium was approached from both directions of reaction only by Rizzi *et al.*[22]

The review of Miller and Smith-Magowan[23] gave $\Delta_r G_m^\circ = 20.2$ kJ·mol^{-1} and $\Delta_r H_m^\circ = -30.7$ kJ·mol^{-1} at $T = 298.15$ K and $I_m = 0.1$ mol·kg^{-1} for the reaction

$$NAD^-(aq) + H_2(g) = NADH^{2-}(aq) + H^+(aq). \quad (15)$$

Adjustment of these quantities for ionic strength as described above yields $\Delta_r G_m^\circ = 22.6_5\ \mathrm{kJ \cdot mol^{-1}}$ and $\Delta_r H_m^\circ = -31.9_4\ \mathrm{kJ \cdot mol^{-1}}$ for reaction (15) at $I = 0$. Combinations of reactions (7), (8), and (9) with the reverse of reaction (15) give the following reactions:

$$\textbf{D}\text{-sorbitol(aq)} = \textbf{D}\text{-fructose(aq)} + H_2(g). \quad (16)$$

$$\textbf{L}\text{-iditol(aq)} = \textbf{L}\text{-sorbose(aq)} + H_2(g), \quad (17)$$

$$\text{xylitol(aq)} = \textbf{D}\text{-xylulose(aq)} + H_2(g). \quad (18)$$

With this thermochemical pathway, the standard reaction quantities for reactions (7), (8), and (9) determined in this study, the aforementioned values of $\Delta_r H_m^\circ$ and $\Delta_r G_m^\circ$ for reaction (15), and the standard formation properties[24] of **D**-fructose(aq), **L**-sorbose(aq), and **D**-xylulose can be used to calculate standard formation properties and standard partial molar entropies for **D**-sorbitol(aq), **L**-iditol(aq), and xylitol(aq). The results of these calculations are given in table 6. As the thermodynamic properties of the pure **D** and **L** forms are equal, these designations have been removed in table 6. Also, the values for these properties are given in table 6 to more significant figures than they are known. This was done to ensure that there were sufficient significant figures so that there would be no loss of accuracy in the calculation of quantities for reactions. The values of the properties of sorbitol(aq), iditol(aq), and xylitol(aq) given in table 6 appear to be the first reported. Also, while an enthalpy of combustion has been reported[25] for xylitol(s), the literature does not appear to contain values for similar quantities for the other two sugar alcohols. Nor do there appear to be any determinations of the third law entropies, solubilities, or enthalpies of solution that are needed to arrive at the formation properties of sorbitol(aq), iditol(aq), and xylitol(aq) via a thermochemical pathway that starts with the elements. However, it is possible to perform some comparisons

TABLE 6. Molar masses M, standard molar enthalpies of formation $\Delta_f H_m^\circ$, standard molar Gibbs free energies of formation $\Delta_f G_m^\circ$, standard partial molar entropies S_m°, and standard partial molar heat capacities $C_{p,m}^\circ$ of the substances pertinent to this study in aqueous solution at $T = 298.15\ \mathrm{K}$ and at $p^\circ = 0.1\ \mathrm{MPa}$. For aqueous solutions, the standard state for the solute is the hypothetical ideal solution ($m = 1\ \mathrm{mol \cdot kg^{-1}}$) and the standard state for the solvent is the pure solvent. The designations **D** and **L** have been removed from these substances, since the thermodynamic properties of the pure forms are equal. The properties of fructose, sorbose, xylose, and xylulose and the values for the standard partial molar heat capacities $C_{p,m}^\circ$ are from reference 24. The properties of iditol, sorbitol, and xylitol were calculated from results obtained in this study

Substance	Formula	M / kg·mol^{-1}	$\Delta_f H_m^\circ$ / kJ·mol^{-1}	$\Delta_f G_m^\circ$ / kJ·mol^{-1}	S_m° / J·K^{-1}·mol^{-1}	$C_{p,m}^\circ$ / J·K^{-1}·mol^{-1}
fructose	$C_6H_{12}O_6$	0.180158	−1259.38	−915.51	279.7	369
iditol	$C_6H_{14}O_6$	0.182174	−1309.94	−938.93	319.4	
sorbitol	$C_6H_{14}O_6$	0.182174	−1312.62	−944.49	328.9	
sorbose	$C_6H_{12}O_6$	0.180158	−1263.30	−911.95	254.6	
xylitol	$C_5H_{12}O_5$	0.152147	−1100.99	−785.23	265.8	
xylose	$C_5H_{10}O_5$	0.150131	−1045.74	−750.49	203.9	279
xylulose	$C_5H_{10}O_5$	0.150131	−1029.65	−746.15	243.3	319

with thermochemical cycle calculations involving ($NADP_{ox}$ + $NADP_{red}$) coupled reactions.

Apparent equilibrium constants have been measured[16,26–28] for the reactions:

$$\text{xylitol(aq)} + \text{NADP}_{ox}\text{(aq)} = \textsc{l}\text{-xylulose(aq)} + \text{NADP}_{red}\text{(aq)}, \quad (19)$$

$$\text{xylitol(aq)} + \text{NADP}_{ox}\text{(aq)} = \textsc{d}\text{-xylose(aq)} + \text{NADP}_{red}\text{(aq)}. \quad (20)$$

Reference reactions that correspond to these respective biochemical reactions are:

$$\text{xylitol(aq)} + \text{NADP}^{3-}\text{(aq)}\ \textsc{l}\text{-xylulose(aq)} + \text{NADPH}^{4-}\text{(aq)} + \text{H}^{+}\text{(aq)}, \quad (21)$$

$$\text{xylitol(aq)} + \text{NADP}^{3-}\text{(aq)}\ \textsc{d}\text{-xylose(aq)} + \text{NADPH}^{4-}\text{(aq)} + \text{H}^{+}\text{(aq)}. \quad (22)$$

As done previously, comparison of results is in terms of calculated values of the equilibrium constants for the reference reactions at $T = 298.15$ K and $I = 0$. Adjustments for ionic strength were made with the extended Debye-Hückel equation with an "ion-size" parameter of 1.6 $kg^{1/2}\cdot mol^{-1/2}$. In performing these calculations, the dissociation constants of the buffers used in these studies were taken from reference 29. Thus, from the results of Hollmann and Touster[26] and Hollmann[16] we calculate respective values of $K_m = 2.0\cdot10^{-11}$ and $K_m = 0.68\cdot10^{-11}$ for the reference reaction (21) at $T = 298.15$ K and $I = 0$. Similarly, from the results of Scher and Horecker[27] and Ditzelmüller *et al.*[28] we calculate respective values of $K_m = 8.5\cdot10^{-11}$ and $K_m = 5.0\cdot10^{-11}$ for the reference reaction (22) at $T = 298.15$ K and $I = 0$. We now use the standard formation properties given in table 6 together with the standard formation properties tabulated by Alberty[32] for $NADP^{3-}$(aq) and for $NADPH^{4-}$(aq) to calculate $K_m = 4.0\cdot10^{-12}$ for the reference reaction (21) and $K_m = 2.3\cdot10^{-11}$ for the reference reaction (22), both at $T = 298.15$ K and $I = 0$. Thus, Hollmann's[16] most recent result (probably preferable to his first[26]) is slightly larger than the value of the equilibrium constant calculated from the formation properties. Similarly, the values of the equilibrium constants calculated from the results of Scher and Horecker[27] and of Ditzelmüller *et al.*[28] are all larger than the value of the equilibrium constant calculated from the formation properties. While measurement error in the apparent equilibrium constants for reactions (19) and (20) or in the formation properties of $NADP^{3-}$(aq) and $NADPH^{4-}$(aq) is the most likely explanation for these differences, another possible explanation could lie in the adjustment of the results to $I = 0$. This problem is exacerbated by the fact that ions with charge numbers of -3 and -4 are reactants in reactions (21) and (22). Also, some of these earlier workers did not specify the compositions of their solutions.

Standard transformed Gibbs free energies of formation $\Delta_f G'^{\circ}_m$ and standard transformed enthalpies of formation $\Delta_f H'^{\circ}_m$ are useful properties for thermodynamic calculations on (overall) biochemical reactions. Specifically, they can be used for biochemical reactions analogous to the way standard formation properties for species are used to calculate standard Gibbs free energies and standard enthalpies of chemical reference reactions. The basis for the calculation of these transformed formation properties has been discussed previously,[30] and the methodology has

TABLE 7. Standard transformed molar enthalpies of formation $\Delta_f H'^{\circ}_m$ and standard transformed molar Gibbs free energies of formation $\Delta_f G'^{\circ}_m$ of aqueous biochemical reactants at $T = 298.15$ K, $p^{\circ} = 0.1$ MPa, pH = 7.0, and $I_m = 0.25$ mol·kg^{-1}. These property values are based on the convention of making a transformation for all of the hydrogen atoms in these substances. The values for NAD_{ox}, NAD_{red}, $NADP_{ox}$, and $NADP_{red}$ are based on the convention $\Delta_f G^{\circ}_m = \Delta_f H^{\circ}_m = 0$ at $I = 0$ for the species NAD^-(aq) and $NADP^{3-}$(aq)

Substance	$\frac{\Delta_f H'^{\circ}_m}{kJ \cdot mol^{-1}}$	$\frac{\Delta_f G'^{\circ}_m}{kJ \cdot mol^{-1}}$
fructose	−1264.30	−426.32
iditol	−1315.68	−368.32
sorbitol	−1318.36	−373.77
sorbose	−1268.22	−422.76
xylitol	−1105.91	−296.04
xylose	−1049.84	−342.83
xylulose	−1033.75	−338.49
NAD_{ox} [a]	−10.26	1059.10
NAD_{red} [a]	−41.38	1120.09
$NADP_{ox}$ [a]	−6.57	1011.86
$NADP_{red}$ [a]	−33.28	1072.95
ATP [b,c]	−2981.79	−2102.88
ADP [b,c]	−2000.19	−1231.48
xylulose 5-phosphate [c,d]		−1219.42

[a] The property values for this reactant are from Alberty.[32]
[b] The property values for this reactant are from Alberty and Goldberg.[31]
[c] The property values for this reactant pertain to pMg = 3.
[d] The calculation of a value of $\Delta_f G'^{\circ}_m$ for this reactant involved the use of an estimated acidity constant and magnesium ion binding constant for xylulose 5-phosphate^{2-}(aq) (see text).

been applied to a number of biochemical reactants.[31–33] Following these earlier papers and the convention[30] of making a transformation for all the hydrogen atoms in these substances, we have used the formation properties given in table 6 to calculate the values of $\Delta_f H'^{\circ}_m$ and $\Delta_f G'^{\circ}_m$ given in table 7. The values of the standard transformed formation properties of the biochemical reactants xylose(aq) and xylulose 5-phosphate(aq) in table 7 were calculated from the standard formation properties of the species which were based on a thermochemical cycle calculation.[24] In the absence of direct measurements, we estimated an acidity constant and Mg^{2+} binding constant for xylulose 5-phosphate^{2-}(aq) from corresponding results[34,35] for ribose 5-phosphate^{2-}(aq). These constants were then used with a value[24] of $\Delta_f G^{\circ}_m$ for the species xylulose 5-phosphate^{2-}(aq) to calculate the value of $\Delta_f G'^{\circ}_m$ for the biochemical reactant xylulose 5-phosphate(aq) given in table 7. Perturbation of both of the aforementioned constants by 50 per cent of their estimated values led to a maximum change of -0.42 kJ·mol^{-1} in the calculated value of $\Delta_f G'^{\circ}_m$ for xylulose 5-phosphate(aq). Thus, we conclude that these estimates do not seriously affect the resulting value of $\Delta_f G'^{\circ}_m$.

We now use the standard transformed formation properties given in table 7 to calculate standard transformed reaction quantities $\Delta_r G'^{\circ}_m$, $\Delta_r H'^{\circ}_m$, and $\Delta_r S'^{\circ}_m$ at $T = 298.15$ K, pH = 7.0, and $I = 0.25$ mol·kg^{-1} for the several reactions given in table 8. In addition to reactions (1), (2), and (3), which were the subject of this

TABLE 8. Standard transformed thermodynamic quantities for several biochemical reactions, including those in the xylose assimilation pathway, at $T = 298.15$ K, pH = 7.0, and $I = 0.25$ mol·kg^{-1}. The values of K' and $\Delta_r G'^{\circ}_m$ for biochemical reaction (25) pertain to pMg = 3. The values of the standard transformed thermodynamic quantities for biochemical reactions (1), (2), (3), (23), and (24) should be satisfactory for these reactions carried out in solutions containing Mg^{2+}(aq)

Biochemical reaction	K'	$\frac{\Delta_r H'^{\circ}_m}{\text{kJ·mol}^{-1}}$	$\frac{\Delta_r G'^{\circ}_m}{\text{kJ·mol}^{-1}}$	$\frac{\Delta_r S'^{\circ}_m}{\text{J·K}^{-1}\text{·mol}^{-1}}$
D-sorbitol(aq) + NAD_{ox}(aq) = D-fructose(aq) + NAD_{red}(aq) [a]	0.033	22.9	8.4	48.6
L-iditol(aq) + NAD_{ox}(aq) = L-sorbose(aq) + NAD_{red}(aq) [b]	0.071	16.3	6.6	32.8
xylitol(aq) + NAD_{ox}(aq) = D-xylulose(aq) + NAD_{red}(aq) [c]	$5.7\cdot10^{-4}$	41.0	18.5	75.5
D-xylose(aq) = D-xylulose(aq) [d]	0.17	16.1	4.3	39.4
D-xylose(aq) + $NADP_{red}$(aq) = xylitol(aq) + $NADP_{ox}$(aq) [e]	0.0031	−29.4	−14.3	−50.5
D-xylulose(aq) + ATP(aq) = D-xylulose 5-phosphate(aq) + ADP(aq) [f]	47		−9.5	

[a] Reaction (1). [b] Reaction (2). [c] Reaction (3). [d] Reaction (23). [e] Reaction (24). [f] Reaction (25).

investigation, reactions (23), (24), and (25) are included in table 8 as they are pertinent to a discussion of the xylose assimilation pathway (see below).

The literature does not appear to contain dissociation constants for the magnesium complexes of NAD^-(aq), $NADH^{2-}$(aq), $NADP^{3-}$(aq), or $NADPH^{4-}$(aq). Alberty,[32] however, has noted that, on the basis of structural similarity, the values of these dissociation constants should be the same for the respective oxidized and reduced forms of these substances. Therefore, the effects due to binding of these substances with Mg^{2+}(aq) should cancel when the respective oxidized and reduced forms are on opposite sides of a reaction. Thus, the use of the values of the standard transformed thermodynamic quantities in table 7 for the calculation of the standard transformed reaction quantities (see table 8) for reactions (1), (2), (3), and (24) is judged to be satisfactory for these reactions carried out in solutions containing Mg^{2+}(aq). Reaction (23) involves neutral species which do not bind Mg^{2+}(aq) and is predicted to be independent of pMg, where $\text{pMg} = -\lg\{c(Mg^{2+})/c^{\circ}\}$. The values given in table 8 for K' and $\Delta_r G'^{\circ}_m$ for reaction (25) pertain to pMg = 3.

The xylose assimilation pathway in bacteria usually proceeds via reactions (23) and (25).[3] The sum of these two reactions is:

$$\text{D-xylose(aq)} + \text{ATP(aq)} = \text{D-xylulose 5-phosphate(aq)} + \text{ADP(aq)}. \qquad (26)$$

In yeast and fungi, the xylose assimilation pathway proceeds via reactions (24), (3), and (25),[3] the sum of which is:

$$\text{D-xylose(aq)} + \text{ATP(aq)} + \text{NADP}_{red}\text{(aq)} + \text{NAD}_{ox}\text{(aq)} =$$
$$\text{D-xylulose 5-phosphate(aq)} + \text{ADP(aq)} + \text{NADP}_{ox}\text{(aq)} + \text{NAD}_{red}\text{(aq)}. \qquad (27)$$

The D-xylulose 5-phosphate(aq) formed from both pathways then enters the pentose phosphate pathway,[3] where further reactions occur. With the values of the standard transformed reaction quantities given in table 8, we obtain, for $T = 298.15$ K, pH = 7.0, pMg = 3.0, and $I = 0.25$ mol·kg^{-1}, $\Delta_r G'^{\circ}_m = -5.2$ kJ·mol^{-1} for reaction (26) (the bacterial pathway) and $\Delta_r G'^{\circ}_m = -5.3$ kJ·mol^{-1} for reaction (27) (the yeast and fungal pathway). Interestingly, the values of $\Delta_r G'^{\circ}_m$ under these near physiological

conditions are essentially the same for both pathways. It is also apparent from the values of $\Delta_r G'^{\circ}_m$ given in table 8 for reactions (3) and (24) that, in the absence of reaction (25), xylitol will be the principal product formed in the yeast and fungal pathway. The primary thermodynamic driving force for both pathways comes from reaction (25) which, to operate properly, is dependent on the existence of an adequate supply of ATP from other metabolic pathways. Clearly the actual operation of the xylose assimilation pathway(s) also involves kinetic and other considerations such as the existence of an adequate supply of cofactors ($NADP_{red}$ and NAD_{ox}) and adequate enzymatic activity.

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