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THERMODYNAMICS OF THE CONVERSION OF AQUEOUS XYLOSE TO XYLULOSE

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The thermodynamics of the conversion of aqueous xylose to xylulose has been investigated using high-pressure liquid chromatography (HPLC) and microcalorimetry. The reaction was carried out in aqueous phosphate buffer over the pH range 6.8–7.4 using solubilized glucose isomerase with $MgSO_4$ as a cofactor. The temperature range over which this reaction was investigated was 298.15–342.15 K. A combined analysis of both the HPLC and microcalorimetric data leads to the following results at 298.15 K for the conversion process: $\Delta G^\circ = 4389 \pm 31$ J mol^{-1}, $\Delta H^\circ = 16090 \pm 670$ J mol^{-1}, and $\Delta C_p^\circ = 40 \pm 23$ J mol^{-1} K^{-1}. The temperature dependence of the equilibrium constant for the reaction is expressed as $R \ln K = -4389/298.15 + 16090[(1/298.15) - (1/T)] + 40[(298.15/T) - 1 + \ln(T/298.15)]$. Comparisons are made with literature data.

1. Introduction

Aqueous xylose can be converted to xylulose using glucose isomerase (EC 5.3.1.5). This process is an intermediate step in the conversion of biomass (hemicellulose) to ethanol and is of potential industrial interest [1,2] in addition to its intrinsic importance to carbohydrate and biological chemistry. Because of the fundamental role of thermodynamics in understanding the nature of chemical reactions, we have undertaken an investigation of this process with an aim towards obtaining a better understanding of its energetics.

In this paper we report the results of both calorimetric measurements, which lead to values of the standard state enthalpy change ΔH° and the heat capacity change ΔC_p° for the conversion of xylose to xylulose over the temperature range 313.15–338.15 K, and high-pressure liquid chromatographic (HPLC) measurements which yield values of the equilibrium constant for this process for the temperature range 298.35–342.15 K. The combined use of these two measurement techniques affords a more complete characterization of the thermodynamics of the process than is obtained from either measurement alone.

2. Experimental

The materials used in this investigation and their sources * were as follows: crystalline D-xylose from Pfanstiehl Laboratories; D-xylulose from Sigma, magnesium nitrate from Fisher Scientific; and potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were Standard Reference Materials Nos. 186-I-c and 186-II-c from NBS. The solubilized glucose isomerase was from *Streptomyces olivaceus*. It was provided by Corn Products Co.

The water content of the xylose and xylulose was carefully determined by Karl Fischer titration

* 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 Bureau of Standards.

and found to be 0.069 and 14.8 mass percent, respectively. These moisture contents were applied as corrections to both the HPLC and the heat measurements.

The analytical information on the glucose isomerase determined by Corn Products on the sample they supplied is as follows: dry substance, 22.3%; moisture, 60.4%; ash, 5.7%; protein (dry basis) by Kjeldahl, 77.8%; activity, 2700 U/g; divalent magnesium, 1.14%; carbohydrate, 1.66%; and 2-propanol, 18.4%. The enzyme sample was dialyzed against 0.5% NaCl solution for 60 h to remove several of the impurities initially present in the enzyme preparation. The NaCl solution was changed several times during the dialysis.

Both the calorimetric and HPLC measurement techniques have been previously described [3]. In these experiments, the flow rate of the mobile phase was 0.4 ml min^{-1}. Three guard columns in series (ODS-5S, ion exclusion, and anion OH) were used to remove the enzyme, cations, and anions during the chromatographic analyses. The retention times of the xylose and xylulose were found to be 19.0 and 22.0 min, respectively. The individual samples of xylose and xylulose showed no impurities when injected into the HPLC. Also, no side products of the reaction were observed following analysis in the HPLC.

As done previously [3], heat measurements were performed by mixing two separate solutions, designated as enzyme and substrate solutions, respectively, in the microcalorimeters. Heats of mixing of enzyme solutions with a (blank) 'substrate' solution containing zero substrate were also determined at each temperature at which measurements were performed: − 1.9 mJ at 313.15 K, − 1.1 mJ at 320.15 K, −0.31 mJ at 325.35 K, − 15.5 mJ at 331.65 K, and − 8.4 mJ at 338.15 K. The scatter in these blank heat effects may be attributable to variations in the homogeneity of the enzyme sample. In any case, they represent a small correction to the measured heats (0.04–2.5%).

Under the conditions used in our microcalorimetric experiments the time required for essentially complete reaction was less than 1 h.

Scanning calorimetric measurements [4] were also performed on aqueous solutions of xylose in phosphate buffer, glucose isomerase with $MgSO_4$ cofactor in phosphate buffer, and both xylose and glucose isomerase in phosphate buffer in order to characterize better the enzyme which was used in our experiment and to determine whether there were any thermal anomalies which needed to be considered in the treatment of the microcalorimetric or the HPLC data. In particular, we were concerned about the scatter in our measured enthalpies of reaction, particularly at 320 K. In all of the scanning experiments the heating rate was 14.8 K per h and the concentrations were typical of those used in the microcalorimetric experiments (see tables 2–5). The experiment involving xylose in buffer showed a single transition centered at 357 K and extending over a 10 degree interval. The glucose isomerase experiment showed both a small broad transition centered at 313 K and a large transition centered at 353 K; the transition at 313 K extended over a 15 degree interval while the one at 353 K extended over a 20 degree interval. The experiment performed where both xylose and glucose isomerase were present showed a peak centered at 359 K and extending over a 10 degree interval. There was no evidence of any thermal anomalies at 320 K.

3. Results and discussion

3.1. Specification of the process

The process of interest to this investigation is the conversion of aqueous xylose to xylulose:

$$\text{xylose(aq)} = \text{xylulose(aq)} \qquad \text{(A)}$$

It is understood that both xylose and xylulose are present in solution as equilibrium mixtures of α and β forms. Our objective is the determination of the standard state Gibbs energy change ($\Delta G°$), enthalpy ($\Delta H°$), and heat capacity ($\Delta C_p°$) changes for process A; the standard state being taken is the usual thermochemical one, namely, the hypothetical ideal solution at unit molality. Isopiestic measurements [5] show that the behavior of aqueous solutions of xylose is very nearly ideal up to about 3 mol kg^{-1} as is to be expected for a nonelectrolyte; similar behavior is predicted for aqueous solutions of xylulose. The first ionization constant

of xylose corresponds to a pK value of 12.22 [6,7]. Thus, if equilibrium or heat measurements are performed at pH values less than 9, ionization corrections become negligible (less than 0.1%). Since the solution behavior of both xylose and xylulose is very nearly ideal and since all of the experiments performed were done at pH values from 6.8 to 7.4, equilibrium constants for process A will be calculated as the ratio of the total stoichiometric molalities of xylose and xylulose in solution and measured enthalpies will be identified with standard state enthalpies of reaction.

Since at equilibrium substantial amounts of both xylose and xylulose are in solution, one can measure the quantity of heat accompanying the conversion of a given number of moles of xylose or xylulose to the equilibrium mixture of the two carbohydrates. Measured heats starting from either side of process A can then be combined [3,8] to yield values of $\Delta H°$ and K for that process.

The molar masses of xylose and xylulose were taken as 150.13 g mol^{-1}.

3.2. HPLC Results

The details of the HPLC results are given in table 1 * and shown in fig. 1. At two temperatures (313.65 and 320.15 K) experiments were also performed starting with xylulose in solution. The results of these experiments are in statistical agreement with the results obtained from those in which only xylose was initially present in solution. The random error (95% confidence limits) is estimated as $\pm$ 0.006 for the entire set of equilibrium measurements; this is twice the pooled estimated standard deviation of the mean for the entire set of measurements.

3.3. Calorimetric Results

The results of the calorimetric experiments are presented in table 2 and are also shown in figs. 1 and 2. In table 2 the apparent enthalpies of reaction of either xylose or xylulose to the equilibrium mixture are designated as $\Delta H'_1$ and $\Delta H'_{-1}$, respectively; i.e. $\Delta H' = -q_{corr}/$(number of moles of substrate) where q_{corr} is the measured heat corrected for 'blank' heat effects. These $\Delta H'$ values are then combined [3] to yield the value of $\Delta H°$ given in column 6 of table 2 and the equilibrium constants (column 7) which one obtained from the ratio $(\Delta H'_1/\Delta H'_{-1})$. The statistical uncertainty (95% confidence limits) associated with the calorimetrically determined $\Delta H°$ values, as a set, is $\pm$ 130 J mol^{-1}. Taking $(\partial \Delta C_p°/\partial T)$ equal to zero leads to $\Delta H° = 16090 \pm 670$ J mol^{-1} and $\Delta C_p° = 40 \pm 23$ J mol^{-1} K^{-1} at 298.15 K using all of the heat measurements. If the data at 320.15 K are excluded from this calculation the results become: $\Delta H° = 16360 \pm 150$ J mol^{-1} and $\Delta C_p° = 33 \pm 8$ J mol^{-1} K^{-1}. In both cases the assigned uncertainties refer to 95% confidence limits. Thus, while the absolute values of $\Delta H°$ and $\Delta C_p°$ are not seriously affected by including the data at 320.15 K the statistical uncertainties are significantly increased.

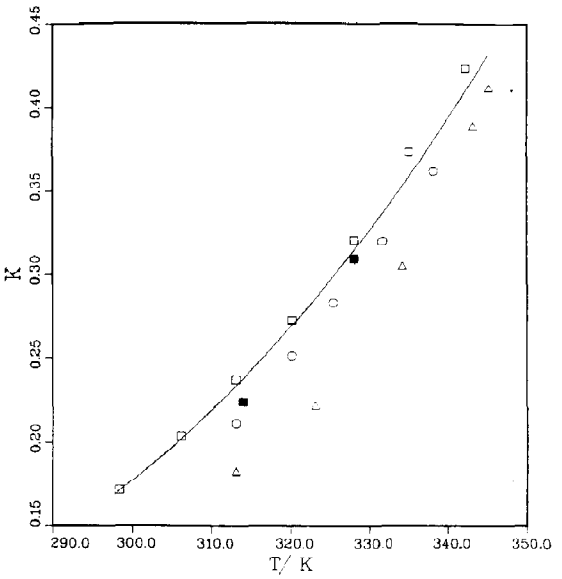

Fig. 1. Equilibrium constants for the conversion of xylose to xylulose as a function of temperature. The open squares are based on the HPLC experiments in which only xylose was present in solution; the solid squares are based on experiments in which only xylulose was present in solution. The octagons were obtained from our calorimetric experiments. The triangles are based upon the liquid chromatography data of Hsiao et al. [1]. Values of K calculated using eq. 1 are shown as the solid line.

* Detailed tables giving the results of individual measurements and available from the authors.

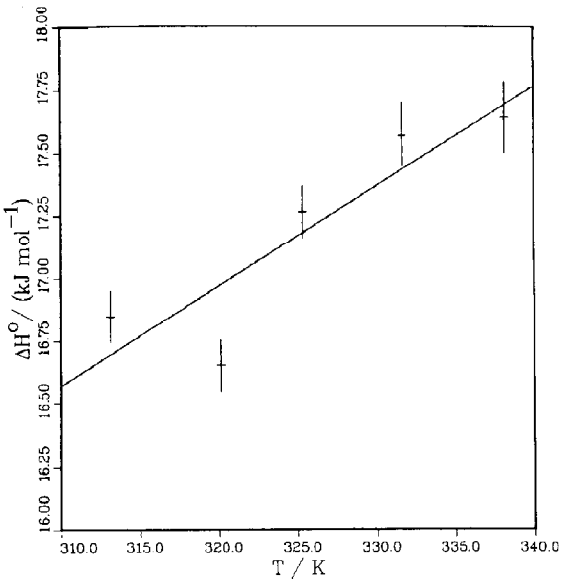

Fig. 2. Enthalpies of conversion of xylose to xylulose as a function of temperature.

Since we have no experimental basis for excluding the measurements at 320.15 K, we prefer the values of $\Delta H° = 16090 \pm 670$ J mol^{-1} and $\Delta C_p° = 40 \pm 23$ J mol^{-1} K^{-1}. The scatter in the enthalpy measurements which leads to these uncertainties is probably attributable to the variable moisture content (i.e., sample inhomogeneity) in the xylulose which is a highly viscous syrup.

Table 1

Equilibrium constants for the conversion of xylose to xylulose determined using HPLC

All of the measurements were done in phosphate buffer at pH 7.4. The concentration of the buffer was 8.7 and 30.3 mmol l^{-1} for KH_2PO_4 and Na_2HPO_4, respectively. The initial concentration of substrate (which was xylose except for those experiments indicated by an asterisk, in which case xylulose was the starting material) was 104–140 mmol (kg solution)$^{-1}$. The enzyme concentration was 99–127 g (kg solution)$^{-1}$ and the $Mg(NO_3)_2$ concentration was 10–15 mmol (kg solution)$^{-1}$. Uncertainties refer to 95% confidence limits.

T (K)	K	No. of measurements
298.35	0.1716 ± 0.0021	6
306.15	0.2038 ± 0.0053	5
313.25	0.2371 ± 0.0028	6
314.05	0.2239 ± 0.0096*	5
320.15	0.2721 ± 0.0065	6
328.15	0.3209 ± 0.0027	8
328.15	0.3090 ± 0.0089*	6
335.05	0.3738 ± 0.0044	6
342.15	0.4236 ± 0.0078	8

3.4. Combined HPLC / microcalorimetry results

Using the values of $\Delta H°$ and $\Delta C_p°$ at 298.15 K for process A obtained above and the equilibrium constants obtained from the HPLC experiments in the model of Clarke and Glew [9] leads to a value of $\Delta G° = 4389 \pm 31$ J mol^{-1} and $K_A = 0.170$ at 298.15 K. If only the equilibrium constants from

Table 2

Enthalpies of isomerization and equilibrium constants for the xylose/xylulose system determined from microcalorimetric measurements

All of the measurements were performed in phosphate buffer at pH 7.4. The concentration of the buffer was 4.22–7.02 and 14.7–24.4 mmol l^{-1} for KH_2PO_4 and Na_2HPO_4, respectively. The enzyme concentration was in the range 165–511 g (kg solution)$^{-1}$ and the $MgSO_4$ concentration of substrate was in the range 95.7–304.9 mmol (kg solution)$^{-1}$. Uncertainties refer to 95% confidence limits.

T (K)	Xylose → equilibrium mixture		Xylulose → equilibrium mixture		$\Delta H°$ (J mol^{-1})	Equilibrium constant (K)
	$\Delta H_1'$ (J mol^{-1})	No. of measurements	$\Delta H_{-1}'$ (J mol^{-1})	No. of measurements		
313.15	2933 ± 75	7	−13914 ± 72	6	16847 ± 104	0.2108 ± 0.0055
320.15	3342 ± 23	8	−13209 ± 110 [a]	6	16551 ± 112	0.2530 ± 0.0027
320.15	–	–	−13410 ± 150 [b]	6	–	–
325.35	3807 ± 78	13	−13456 ± 58	5	17263 ± 97	0.2829 ± 0.0059
331.65	4264 ± 53	8	−13305 ± 115	6	17569 ± 127	0.3205 ± 0.0049
338.15	4684 ± 54	5	−12956 ± 131	6	17640 ± 142	0.3615 ± 0.0055

[a] Series 1.
[b] Series 2.

the HPLC experiments are used the following values are obtained: $\Delta G° = 4379 \pm 43$ J mol^{-1}, $\Delta H° = 16316 \pm 1440$ J mol^{-1}, and $\Delta C_p° = 60 \pm 65$ J mol^{-1} K^{-1}. Thus, while the values of $\Delta H°$ and $\Delta C_p°$ calculated from the equilibrium constants above are in agreement with the calorimetric values, they are not as precise. In summary, we adopt for process A at 298.15 K: $\Delta G° = 4389 \pm 31$ J mol^{-1}, $\Delta H° = 16090 \pm 670$ J mol^{-1}, and $\Delta C_p° = 40 \pm 23$ J mol^{-1} K^{-1}. We believe that the assigned uncertainties are adequate to account for both random and systematic errors in the measurements.

The values obtained above can be introduced into the equation of Clarke and Glew [9] to yield the following equations for the temperature dependence of the equilibrium constant:

$$R\ln K = -4389/298.15 + 16090[(1/298.15) - (1/T)] + 40[(298.15/T) - 1 + \ln(T/298.15)]. \quad (1)$$

where R is the gas constant (8.31441 J mol^{-1} K^{-1}) and T the absolute temperature. Values of K calculated using the above equation are shown as the solid line in fig. 1.

3.5. Comparisons with literature data

The only other data in the literature which lead to the equilibrium constant or enthalpy change for process A are the data of Hsiao et al. [1]. The equilibrium constants calculated from the percent conversion data in table 1 of their paper are shown in fig. 1. Fixing $\Delta C_p°$ at 40 J mol^{-1} K^{-1} and using the equilibrium constants calculated from their data lead to $\Delta G° = 5372 \pm 392$ J mol^{-1} and $\Delta H° = 22.3 \pm 3.7$ kJ mol^{-1} for process A at 298.15 K. These values are not in agreement with our measurements. Neither the method of measurement (liquid chromatography) nor the conditions of measurement (1 mol l^{-1} D-xylose, 0.001 mol l^{-1} $MgSO_4$, 0.05% sodium bisulfite, in 0.1 mol l^{-1} β-glycerophosphate buffer at pH 7.5 using immobilized glucose isomerase) offers any obvious clues as to why their results should differ from ours.

The available thermochemical data in the literature on D-xylose are enthalpies of combustion of the crystal [10], enthalpies of solution as a function of temperature [11], enthalpies of mutarotation in aqueous solution [12], enthalpies of ionization [6], ionization constants [6,7], and activity and osmotic coefficients [5]. Other than the data of Hsiao et al. [1] and the measurements reported herein there are no thermochemical data in the literature on xylulose.

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