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THERMODYNAMICS OF CARBOHYDRATE ISOMERIZATION REACTIONS

THE CONVERSION OF AQUEOUS ALLOSE TO PSICOSE

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The thermodynamics of the conversion of aqueous D-psicose to D-allose has been investigated using high-pressure liquid chromatography. The reaction was carried out in phosphate buffer at pH 7.4 over the temperature range 317.25–349.25 K. The following results are obtained for the conversion process at 298.15 K: $\Delta G^\circ = -1.41 \pm 0.09$ kJ mol^{-1}, $\Delta H^\circ = 7.42 \pm 1.7$ kJ mol^{-1}, and $\Delta C_p^\circ = 67 \pm 50$ J mol^{-1} K^{-1}. An approximate equilibrium constant of 0.30 is obtained at 333.15 K for the conversion of aqueous D-psicose to D-altrose. Available thermodynamic data for isomerization reactions involving aldohexoses and aldopentoses are summarized.

1. Introduction

This paper is the fourth in a series of studies of the thermodynamics of the isomerization of carbohydrates using the enzyme glucose isomerase (EC 5.3.1.5). Earlier studies dealt with the isomerization of glucose to fructose [1], xylose to xylulose [2], and ribose to ribulose and to arabinose [3]. We report herein the results of a study of the thermodynamics of conversion of allose * to psicose:

$$\text{allose(aq)} = \text{psicose(aq)} \qquad \text{(A)}$$

in which equilibrium constants were determined from 44.1 to 76.1°C using high-pressure liquid chromatography (HPLC).

Since the existence of the above reaction had apparently not been previously known, the existence of other possible carbohydrate isomerization reactions using glucose isomerase was also investigated.

2. Experimental

The materials used in this investigation and their sources * were: psicose, a viscous syrup, from Pfanstiehl; allose(c) and altrose(c) from Sigma; $Mg(NO_3)_2$ from Fisher Scientific; and KH_2PO_4 and Na_2HPO_4 were Standard Reference Materials nos. 186-I-c and 186-II-c from the National Bureau of Standards. The solubilized glucose isomerase was from *Streptomyces olivochromogenes* and was provided by Corn Products.

Information on the characterization of the glu-

* Only the D forms of carbohydrates were used in this study and in our earlier studies. Therefore, the prefix D will be implicitly understood when referring to carbohydrates in this paper.

* 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.

cose isomerase is contained in other publications [1,2,4]. To characterize the enzyme further, two-dimensional, denaturing gel-electrophoresis experiments were performed. These experiments indicated the presence of albumin in the sample and showed two segments identical in molecular mass (~ 40 kda) at pH 5.0 and 7.5. These two segments were approx. 20 charge units apart.

The moisture contents of the psicose and allose were determined by Karl Fischer titration and found to be 7.4 and 0.12 mass percent, respectively. These moisture contents were applied as corrections to the HPLC measurements.

The HPLC methods have been previously described [1,2]. The retention times of altrose, allose and psicose were 13.4, 16.9 and 21.2 min, respectively at a flow rate of 0.6 ml min^{-1}. HPLC analysis of allose showed no impurities in the sample used. However, HPLC analysis of psicose showed that it contained fructose in the amount of 6.3 mass percent. A correction for this fructose impurity was applied in the determination of the response factor of psicose.

Solutions containing only allose or psicose were prepared in phosphate buffer (8.7 mmol l^{-1} for KH_2PO_4 and 30.3 mol l^{-1} for Na_2HPO_4) at pH 7.4 containing glucose isomerase and allowed to equilibrate for 4–7 days prior to analysis. Since the equilibration times were this long, no calorimetric experiments were feasible.

It was also found that a small amount of altrose (about 2.5 mol% of the total amount of the carbohydrates present in solution) was formed at 333.15 K after a 7 day period. Because of this finding, two additional experiments were performed at the same temperature over a 14 day period. In the first experiment only altrose was present at the initiation of the reaction and in the second only allose was present at the start. It was found that the ratios of the concentrations of altrose to psicose determined in these two separate experiments differed by a factor of two. This indicated that the system had not yet reached equilibrium. Since experiments longer than 14 days were not practical, no further attempts were made to determine a precise equilibrium constant for the conversion of psicose to altrose. However, based upon these approximate experiments, the equilibrium constant for the conversion of psicose to altrose is in the range 0.20–0.40 at 333.15 K.

We also investigated the possibility of other isomerization reactions involving the following hexoses; sorbose, galactose, tagatose and talose. In each case, a sample of the hexose was allowed to equilibrate for 2 weeks at 333.15 K in an aqueous phosphate buffer (pH 7.4) which contained glucose isomerase at the same concentration as used for studying the conversion of allose to psicose. Analysis of the reaction mixtures showed no evidence of aldo-keto conversion.

3. Results and discussion

The results of the HPLC experiments are given in table 1. Note that the equilibrium constants (K) determined starting from either pure allose or pure psicose are in agreement. This is both consistent and highly indicative of the existence of a true equilibrium in the reaction mixture. The data are interpreted in terms of the following equation:

$$R \ln K = -\Delta G^\circ/298.15 + \Delta H^\circ[(1/298.15) - (1/T)] + \Delta C_p^\circ[(298.15/T) - 1 + \ln(T/298.15)] \quad (1)$$

In the above equation ΔG°, ΔH° and ΔC_p° are the standard state Gibbs energy, enthalpy and

Table 1

Equilibrium constants for the conversion of aqueous allose to psicose determined using HPLC

All of the measurements were performed in phosphate buffer (8.7 mmol l^{-1} for KH_2PO_4 and 30.3 mmol l^{-1} for Na_2HPO_4) at pH 7.4. The initial concentration of substrate was 50–80 mmol (kg solution)$^{-1}$. Equilibration times were 4–7 days. The enzyme concentration was ~ 150 g (kg solution)$^{-1}$. All uncertainties refer to 95% confidence limits.

T(K)	K (starting from allose)	K (starting from psicose)	K (average)
317.25	2.179 ± 0.031	2.120 ± 0.039	2.15
325.15	2.339 ± 0.027	2.298 ± 0.011	2.32
333.15	2.651 ± 0.041	2.543 ± 0.048	2.55
341.55	2.769 ± 0.038	2.772 ± 0.044	2.77
349.25	3.011 ± 0.028	3.006 ± 0.024	3.01

heat capacity changes for process A, T the thermodynamic temperature, and R the gas constant (8.31441 J mol^{-1} K^{-1}). Eq. 1 can be derived from the Second Law of Thermodynamics and it is identical to the model of Clarke and Glew [5] when ΔC_p° is temperature invariant.

Fitting the experimental data to eq. 1 leads to the following; $\Delta G^\circ = -1.40 \pm 0.16$ kJ mol^{-1}, $\Delta H^\circ = 7.7 \pm 3.1$ kJ mol^{-1} and $\Delta C_p^\circ = 59 \pm 90$ J mol^{-1} K^{-1} for process A at 298.15 K. If ΔC_p° is fixed at 75 J mol^{-1} K^{-1} based upon data for an analogous reaction, the conversion of glucose to fructose [1], the following values are calculated: $\Delta G^\circ = -1.42 \pm 0.04$ kJ mol^{-1} and $\Delta H^\circ = 7.15 \pm 0.36$ kJ mol^{-1} for process A at 298.15 K. The uncertainties in both of the above cases refer to 95% confidence limits. Note that the uncertainty both in ΔG° and, in particular, in ΔH° is substantially improved if ΔC_p° is well known. This is not surprising and it serves to emphasize the need for an accurate value of ΔC_p°.

Since the estimated value of ΔC_p° may be as reliable as the one determined from the variation of the equilibrium constant with temperature and since it and the calculated value are approximately the same, we adopt a value of $\Delta C_p^\circ = 67 \pm 50$ J mol^{-1} K^{-1} (the average of 59 and 75 J mol^{-1} K^{-1}) for process A. Using this value and the experimental equilibrium data we calculate $\Delta G^\circ = 1.41 \pm 0.09$ kJ mol^{-1} and $\Delta H^\circ = 7.4_2 \pm 1.7$ kJ mol^{-1} for process A at 298.15 K. Here the uncertainties were obtained by varying the value of ΔC_p° by the assigned uncertainty of ± 50 J mol^{-1} K^{-1}.

The experimentally determined values of K_A are shown in fig. 1 together with values calculated using eq. 1 and the above set of thermodynamic parameters. There do not appear to be any thermochemical data in the literature on this system with which to compare these results.

The available information on the thermodynamics of the aldo-keto isomerization of hexoses and pentoses is summarized in table 2. The Gibbs energy change for the conversion of psicose to altrose is based upon the approximate equilibrium constant of 0.3 determined at 333.15 K which was then adjusted to yield a value of 0.25 at 298.15 K using the enthalpy and heat capacity changes for the conversion of glucose to fructose. The Gibbs energy change for the conversion of allose to altrose is obtained by combining the Gibbs energy changes for the conversion of allose to psicose and of psicose to altrose. Thus, for the conversion of aqueous allose to altrose the equilibrium constant is approx. 0.45 at 298.15 K.

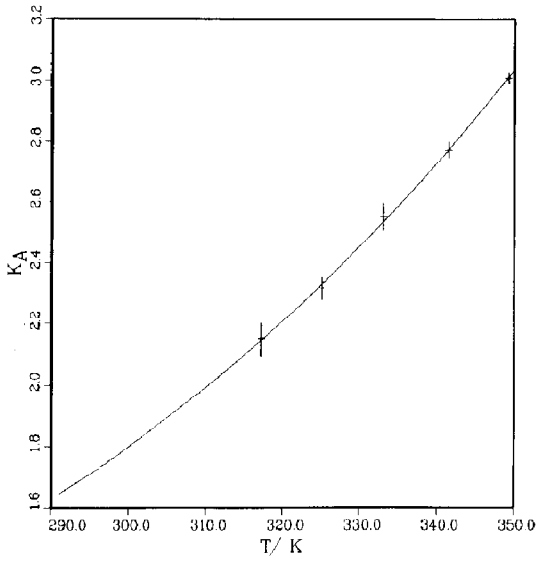

Fig. 1. Equilibrium constants (K_A) for the conversion of aqueous allose to psicose as a function of temperature. The individual points are the experimental values determined using HPLC. The solid line was calculated using eq. 1 with $\Delta G^\circ = -1.41$ kJ mol^{-1}, $\Delta H^\circ = 7.42$ kJ mol^{-1} and $\Delta C_p^\circ = 67$ J mol^{-1} K^{-1}.

With the exception of the data for the mannose isomerase catalyzed conversion of mannose to fructose [6] all of the results in table 2 were determined in our laboratory using both HPLC and, where feasible, calorimetry to determine both the enthalpy and heat capacity changes. The data for the conversion of glucose to mannose were calculated using the data for the conversions of glucose to fructose and of fructose to mannose.

The aldohexoses and aldopentoses exist as a mixture of furanose and pyranose structures and in α and β forms [7]. While the enthalpy difference between the α and β forms in aqueous solution is not large [8,9], i.e., 1–2 kJ mol^{-1}, the enthalpy difference between furanose and

Table 2

Thermodynamics of isomerization reactions involving D-aldohexoses and D-aldopentoses in aqueous solution 298.15 K

Process	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔC_p° (J mol^{-1} K^{-1})	References
Ribose = arabinose	-3.44 ± 0.30	-9.8 ± 3.0 [a]	-21.3		[3]
Ribose = ribulose	$2.85 + 0.14$	$11.0 + 1.5$ [a]	27.3		[3]
Xylose = xylulose	$4.38_9 \pm 0.031$	16.09 ± 0.67 [a]	39.2	40 ± 23 [c]	[2]
Arabinose = ribulose	6.29 ± 0.34	20.75 ± 3.4 [a]	48.5		[3]
Allose = psicose	-1.41 ± 0.09	$7.4_2 \pm 1.7$ [b]	29.6	67 ± 50 [d]	this paper
Psicose = altrose	~ 3.4				this paper
Allose = altrose	~ 2.0				calculated
Glucose = fructose	0.349 ± 0.053	2.78 ± 0.20 [a]	8.15	76 ± 30 [c]	[1]
Mannose = fructose	-2.72	0.0 [b]	9.12		[6]
Glucose = mannose	3.07	2.78	-0.97		calculated

[a] Based on direct calorimetric measurements.
[b] Calculated from the temperature dependency of equilibrium constants.
[c] Calculated from the temperature dependency of enthalpies of reaction.
[d] Based on both an estimate and the temperature dependency of equilibrium constants.

pyranose structures is a large quantity (~ 12 kJ mol^{-1}) [10]. These structural and energetic considerations serve to complicate a detailed intercomparison of the overall thermodynamic results given in table 2 and make difficult the possible development of a predictive scheme which would yield reliable thermodynamic parameters for isomerization reactions which have not yet been studied. Nevertheless, there is a structural similarity between the ribose/ribulose isomerization and the aldose/psicose isomerization which is reflected in entropy changes that are very close to each other. However, a similar intercomparison between the xylose/xylulose and glucose/fructose isomerization shows entropy changes that differ by 31 J mol^{-1} K^{-1}. It would appear that a more complete understanding of the thermodynamics of these interconversions requires at least a detailed understanding of the thermodynamics of the furanose/pyranose interconversions, possibly additional information on the α to β conversions, and also additional data on other isomerization reactions. Nevertheless, the thermodynamic parameters given in table 2 represent the most complete set of information obtained on the thermodynamics of carbohydrate isomerization reactions to date.

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