A thermodynamic investigation of the hydrolysis of sucrose to fructose and glucose has been performed using microcalorimetry and high-pressure liquid chromatography. The calorimetric measurements were carried out over the temperature range 298–316 K and in sodium acetate buffer (0.1 M, pH 5.65). Enthalpy and heat capacity changes were obtained for the hydrolysis of aqueous sucrose (process A): sucrose(aq) + H₂O(l) = glucose(aq) + fructose(aq). The determination of the equilibrium constant required the use of a thermochemical cycle calculation involving the following processes: (B) glucose 1-phosphate²⁻ (aq) = glucose 6-phosphate²⁻ (aq); (C) sucrose(aq) + HPO₄²⁻ (aq) = glucose 1-phosphate²⁻ (aq) + fructose(aq); and (D) glucose 6-phosphate²⁻ (aq) + H₂O(l) = glucose(aq) + HPO₄²⁻ (aq). The equilibrium constants determined at 298.15 K for processes B and C are 17.1 ± 1.0 and 32.4 ± 3.0, respectively. Equilibrium data for process D was obtained from the literature, and in conjunction with the data for processes B and C, used to calculate a value of the equilibrium constant for the hydrolysis of aqueous sucrose. Thus, for process A, ΔG° = −26.53 ± 0.30 kJ mol⁻¹, K° = (4.44 ± 0.54) × 10⁶, ΔH° = −14.93 ± 0.16 kJ mol⁻¹, ΔS° = 38.9 ± 1.2 J mol⁻¹ K⁻¹, and ΔC° = 57 ± 1.4 J mol⁻¹ K⁻¹ at 298.15 K. Additional thermoregulatory conditions that bear upon the accuracy of these results are examined.

The hydrolysis of sucrose to glucose and fructose is catalyzed by the enzyme β-fructofuranosidase (EC 3.2.1.26) which is also called invertase. Although the enthalpy of hydrolysis has been studied by earlier workers (1–5), the determination of the equilibrium constant for this reaction remains a difficult problem because the direction of reaction lies almost completely in the direction of the formation of glucose and fructose. Consequently, it is necessary to determine equilibrium constants (i.e. Gibbs energy changes) for a series of reactions which can be algebraically combined to yield a value of the equilibrium constant for the hydrolysis reaction. The three reactions which we have used for this purpose are: 1) the conversion of glucose 1-phosphate to glucose 6-phosphate, 2) the reaction of sucrose with inorganic phosphate to form glucose 1-phosphate and fructose, and 3) the hydrolysis of glucose 6-phosphate to glucose and inorganic phosphate.

This study is a continuation of earlier work (6, 7) on the hydrolysis of disaccharides which had indicated that the entropy changes for the hydrolysis of disaccharides were reasonably constant and ranged from 30 to 35 J mol⁻¹ K⁻¹. Whether this rule extended to other disaccharide linkages was an additional motivation for this study. The data obtained in this study provide essentially a complete characterization of the thermodynamics of hydrolysis of sucrose.

EXPERIMENTAL PROCEDURES

The materials used in this study and their sources are: glucose 1-phosphate, glucose 6-phosphate, β-fructofuranosidase, sucrose phosphorylase (EC 2.4.1.7), and phosphoglucomutase (EC 5.4.2.2) were obtained from Sigma; the glucose, sucrose, and disodium hydrogen phosphate are Standard Reference Materials from the National Bureau of Standards; the Tris and the sodium acetate buffers were from Fisher and Baker, respectively. The moisture contents of the substrates as determined by Karl Fischer titration were, in mass % of water: glucose 1-phosphate, 9.2%; glucose 6-phosphate, 17.8%; fructose, 0.3%; glucose, 0.03%; and sucrose, 0.02%. Corrections for these moisture contents were applied to both the calorimetric and equilibrium data. The substrates were found to be pure using the chromatographic arrangements described below.

In the sucrose hydrolysis experiments, measurements of the amounts of the substrates in solution were performed using a Dionex Bio-LC (HPIC-AS6 anion exchange column, pulsed amperometric detector, 0.05 M aqueous sodium hydroxide mobile phase, flow rate of 0.7 ml/min). Typical retention times of the carbohydrates were 8 min for glucose and fructose and 18.3 min for sucrose. A similar chromatographic arrangement (Dionex Bio-LC, HPIC-AS6 column, pulsed amperometric detector, mobile phase consisting of 0.1 M NaOH and 1.0 M sodium acetate, flow rate of 0.8 ml/min) was used for the studies involving the conversion of glucose 1-phosphate to glucose 6-phosphate. The glucose 1-phosphate and glucose 6-phosphate had retention times of 6.5 and 17 min, respectively. In the experiments involving the reaction of sucrose with inorganic phosphate to form glucose 1-phosphate and fructose, it was necessary to use two different chromatographs. Sucrose was determined as described above using the Dionex Bio-LC. The Dionex Bio-LC (HPIC-AS6 column, pulsed amperometric detector, mobile phase consisting of 0.1 M NaOH and 0.18 M sodium acetate, flow rate of 0.8 ml/min) was also used for the measurement of glucose 1-phosphate concentrations. The fructose concentration was determined using a Hewlett-Packard HP-1090 liquid chromatograph (Bio-Rad HFX-87C calcium cation exchange column, refractive index detector, pure water mobile phase, flow rate of 0.6 ml/min). The retention time of the fructose was 15 min. The amount of inorganic phosphate present was determined using the Dionex Bio-LC (Ion Pac AS2 column, conductivity detector, 0.05 M NaOH mobile phase, flow rate of 1.0 ml/min). Under these conditions, the retention time of inorganic phosphate was 4.3 min. Equilibrations of solutions were carried out with gentle stirring in a thermostatted water bath. As in the previous study (7), equilibrium was approached from both a forward and reverse direction. It was found that chemical equilibrium, as evidenced by the agreement of equilibrium ratios determined from both the forward and reverse directions, was attained within 1 day for the equilibrium involving glucose 1-phosphate and glucose 6-phosphate. Four days were allowed for the study involving the equilibrium between sucrose, inorganic phosphate, fructose, and glucose 1-phosphate. Here, the equilibrium ratios determined from both the forward and reverse directions differed from each other by only a small amount outside their respective 95% confidence limits. There were no interferences from the enzymes in the chromatographic measurements.

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Thermodynamics of the Hydrolysis of Sucrose*

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Thermodynamics of Sucrose Hydrolysis

The calorimetric procedures are similar to those used previously (8, 9). Control experiments of the same type used in the previous investigation (7) yielded a combined correction of \(-0.68 \pm 1.11\) mJ to be applied to the measured heats of reaction which were in the range 380–500 mJ. Chromatographic analyses of the final calorimetric reaction mixtures showed that in all cases the hydrolysis of sucrose was complete (>99.98%) within 30 min of its initiation.

RESULTS AND DISCUSSION

The primary process of interest in this study is the hydrolysis of sucrose to glucose and fructose.

Sucrose(aq) + H₂O(liq) = glucose(aq) + fructose(aq) (A)

Since at equilibrium there is very little sucrose left, it is necessary to obtain the equilibrium constant for this process by appropriate combination of the equilibrium constants or Gibbs energy changes for the following processes.

Glucose 1-phosphate\(^{-}\)(aq) = glucose 6-phosphate\(^{-}\)(aq) (B)

Sucrose(aq) + HPO\(^{3-}\)(aq) = glucose 1-phosphate\(^{-}\)(aq) + fructose(aq) (C)

Glucose 6-phosphate\(^{-}\)(aq) + H₂O(liq) = glucose(aq) + HPO\(^{3-}\)(aq) (D)

The Gibbs energy change (\(\Delta G^\circ\)) and equilibrium constant (\(K\)) for process A can be calculated:

\[ \Delta G^\circ_A = \Delta G^\circ_B + \Delta G^\circ_C + \Delta G^\circ_D = -RT \log K_A \] (1)

Here, \(R\) is the gas constant and \(T\) is the thermodynamic temperature. The standard states and the model used to predict the temperature dependence of equilibrium constants and enthalpies of reaction have been previously discussed (7). Note also that hydrolysis reactions involving non-electrolytes will have a negligible dependence on pH over a range that is well removed from the pK values for the ionization of the reactant or product(s) and should have a negligible dependence on ionic strength.

The results of equilibrium measurements on processes B and C are given in Table I. For the reaction catalyzed by phosphoglucomutase, the equilibrium ratios determined from both the forward and reverse directions are within experimental error, in agreement with each other. The forward and reverse direction equilibrium ratios for the reaction catalyzed by sucrose phosphorylase differ, however, by a small amount outside the 95% confidence limits. In both cases we have averaged the results to obtain apparent equilibrium constants of 17.10 ± 1.0 and 32.4 ± 3.0 for processes B and C, respectively, at 298.15 K. The adjustment to the standard state for the reaction catalyzed by phosphoglucomutase is particularly small (0.02) since the ionization constants of glucose 1-phosphate and glucose 6-phosphate are almost identical. The adjustment of 0.9 applied to the reaction catalyzed by sucrose phosphorylase is due to the difference in ionization constants between glucose 1-phosphate and inorganic phosphate. The uncertainties assigned to the equilibrium constants have also been slightly increased from the 95% confidence limits obtained from the random errors in the equilibrium measurements. The corresponding Gibbs energy changes for these processes are \(-7.04 \pm 0.15\) and \(-8.62 \pm 0.23\) kJ mol\(^{-1}\) for processes B and C, respectively. For process D, we adopt a value of the Gibbs energy change at 298.15 K equal to \(-10.871 \pm 0.10\) kJ mol\(^{-1}\) from our earlier review (12). This value is primarily based upon measurements of Lawson and Veech (14). Use of these values in Equation 1 leads to a Gibbs energy change of \(-26.53 \pm 0.30\) kJ mol\(^{-1}\) for process A, the hydrolysis of sucrose. The equilibrium constant is calculated to be \((4.44 \pm 0.54) \times 10^4\) at 298.15 K.

The results of the calorimetric measurements are given in Table II and shown in Fig. 1. A least squares fit to the data yield a value of \(H^\circ_A = -14.93 \pm 0.16\) kJ mol\(^{-1}\) and \(C^\circ_A = 57 \pm 14\) J mol\(^{-1}\) K\(^{-1}\) for process A, the hydrolysis of sucrose. The uncertainties are 95% confidence limits obtained from the least squares fit. Combination of this calorimetrically determined enthalpy change with the Gibbs energy change obtained from the thermochemical cycle leads to a value of 38.9 ± 1.2 J mol\(^{-1}\) K\(^{-1}\) for the entropy of hydrolysis of sucrose at 298.15 K. This entropy change falls into the same range of values (30–43 J mol\(^{-1}\) K\(^{-1}\)) found for the hydrolysis of other disaccharides (6, 7). Since values of the equilibrium constant, Gibbs energy, enthalpy, and heat capacity changes have been obtained for the hydrolysis reaction, Equations 2 and 3 in the previous paper (7) can be used to predict the temperature dependence of these quantities for this process.

Turning to an examination of data in the literature with which our measurements can be compared, we first consider the equilibrium data pertinent to the reactions catalyzed by phosphoglucomutase and sucrose phosphorylase (processes B and C, respectively). Our result of 17.1 ± 1.0 for the equilib-

<table>
<thead>
<tr>
<th>Direction of reaction</th>
<th>[Glu-1-P]</th>
<th>[Glu-6-P]</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Glu-1-P</td>
<td>0.3171 ± 0.013</td>
<td>5.279 ± 0.092</td>
<td>16.65 ± 0.74</td>
</tr>
<tr>
<td>From Glu-6-P</td>
<td>0.3223 ± 0.0071</td>
<td>5.668 ± 0.051</td>
<td>17.59 ± 0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Sucrose]</th>
<th>[P]</th>
<th>[Glu-1-P]</th>
<th>Fructose</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>From sucrose and P</td>
<td>5.221 ± 0.077</td>
<td>11.57 ± 0.37</td>
<td>38.84 ± 0.61</td>
<td>46.01 ± 2.6</td>
</tr>
<tr>
<td>From fructose and Glu-1-P</td>
<td>5.820 ± 0.19</td>
<td>9.318 ± 0.15</td>
<td>38.23 ± 0.52</td>
<td>46.17 ± 0.52</td>
</tr>
</tbody>
</table>
for the hydrolysis reaction obtained in this study and in kJ mol\(^{-1}\), are: \(-14.61 \pm 0.09\) from Barry (1); \(-17.66 \pm 0.22\) from Kozaki (2); \(-14.91 \pm 0.12\) from Sturtevant (3); \(-14.12 \pm 0.20\) from Bauer and Gemmill (4); and \(-12.73 \pm 0.34\) from Lazniewski (5). Kozaki (2) and Bauer and Gemmill (4) used \(\beta\)-fructofuranosidase while the other workers used acid catalysis to bring about the hydrolysis reaction. Our result of \(-14.93 \pm 0.16\) kJ mol\(^{-1}\) is in excellent agreement with the earlier, careful measurements of Sturtevant (3). None of the previous workers investigated the temperature dependence of the reaction.

The enthalpy change for the hydrolysis of sucrose (process A) can also be compared with the value calculated using the enthalpy of combustion (19) and enthalpy of solution (20) of sucrose(cr) and the enthalpies of formation of aqueous glucose and fructose (12). The resulting value is \(-16.04 \pm 1.5\) kJ mol\(^{-1}\). It is in agreement with the direct measurement of \(-14.93 \pm 0.16\) kJ mol\(^{-1}\) obtained in this study. The value of the enthalpy of combustion of sucrose is particularly well established in the literature (19, 21). The enthalpy of solution of sucrose (5.76 kJ mol\(^{-1}\)) determined by Jasra and Ahluwalia (20) and used in this calculation is in good agreement with the earlier measurement (6.1 kJ mol\(^{-1}\)) of Higbie and Stegeman (22), but not those of Barry (1) or Hendricks et al. (23) (4.9 and 5.0 kJ mol\(^{-1}\), respectively).

Parks et al. (24) report a third law entropy for sucrose(cr). This entropy is combined with the solubility of sucrose(cr) and its activity coefficient at saturation (25), as determined by Scatchard et al. (26), and the enthalpy data (19, 20) to obtain the Gibbs energy of formation of sucrose(aq). This Gibbs energy of formation is then combined with the formation properties of glucose(aq) and fructose(aq) (12) to calculate the Gibbs energy change for the hydrolysis of sucrose. The result of this calculation is \(\Delta G_f^\circ = -39.3\) kJ mol\(^{-1}\) at 298.15 K. It differs from the result of \(-26.53 \pm 0.30\) kJ mol\(^{-1}\) obtained in this investigation. Since the thermodynamic cycle calculation using the enthalpies yielded a result in agreement with our direct measurement, this difference is assigned to cumulative errors of \(\approx 42\) kJ mol\(^{-1}\) K\(^{-1}\) in the entropies of sucrose, glucose, and fructose. New determinations of the third law entropies of these three compounds would be particularly valuable. It would not only serve to resolve this discrepancy, but it could also provide a firm foundation for the formation properties of these substances which enter into several thermochemical and metabolic pathways (12, 26, 27).

The final thermochemical cycle calculation to be considered uses the partial molar heat capacities (\(C_{p,M}\)) of aqueous sucrose, glucose, and fructose. The partial molar heat capacity of sucrose (633 J mol\(^{-1}\) K\(^{-1}\) at 298.15 K) is accurately known from the very careful measurements of Gucker and Ayres (28). Other workers (20, 29, 30) have made measurements from which results ranging from 624 to 650 J mol\(^{-1}\) K\(^{-1}\) are obtained for the partial molar heat capacity of aqueous sucrose. Use of the partial molar heat capacities of aqueous glucose and fructose from our review (12) leads, together with the result of Gucker and Ayres (28), to \(\Delta C_{p,M} = -3 \pm 20\) J mol\(^{-1}\) K\(^{-1}\) for the hydrolysis of aqueous sucrose. Our result, obtained from the temperature dependence of enthalpies of hydrolysis, was \(57 \pm 14\) J mol\(^{-1}\) K\(^{-1}\). We believe that the discrepancy is attributable to errors in the measurements leading to the partial molar heat capacity of aqueous fructose. The following calculations make this point. Since the partial molar heat capacity of glucose (336 J mol\(^{-1}\) K\(^{-1}\)) appears to be known (12) more accurately than that of aqueous fructose, we assume it and the heat capacity change of \(57 \pm 14\) J mol\(^{-1}\) K\(^{-1}\) determined herein to be correct and combine it with the partial molar heat capacity of sucrose determined by Gucker.
and Ayres (28) to calculate a value of \( 429 \pm 16 \) J mol\(^{-1}\) K\(^{-1}\) for aqueous fructose. Use of the value of \( \Delta C_p \) of \( 76 \pm 30 \) J mol\(^{-1}\) K\(^{-1}\) reported (31) for the conversion of aqueous glucose to fructose together with the partial molar heat capacity of aqueous glucose leads to \( C_{p2} = 412 \pm 32 \) J mol\(^{-1}\) K\(^{-1}\) for aqueous fructose. Thus, these two pathways lead to a value of \( C_{p2} \) for aqueous fructose which are in agreement and the discrepancy appears to be largely resolved. Again, a direct and careful determination of the partial molar heat capacity of aqueous fructose would be useful in firmly establishing its value.

REFERENCES