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Thermodynamics of Hydrolysis of Disaccharides

CELLOBIOSE, GENTIOBIOSE, ISOMALTOSE, AND MALTOSE*

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The thermodynamics of the enzymatic hydrolysis of cellobiose, gentiobiose, isomaltose, and maltose have been studied using both high pressure liquid chromatography and microcalorimetry. The hydrolysis reactions were carried out in aqueous sodium acetate buffer at a pH of 5.65 and over the temperature range of 286 to 316 K using the enzymes β-glucosidase, isomaltase, and maltase. The thermodynamic parameters obtained for the hydrolysis reactions, disaccharide(aq) + H_2O(liq) = 2 glucose(aq), at 298.15 K are: $K \geq 155$, $\Delta G^0 \leq -12.5$ kJ mol^{-1}, and $\Delta H^0 = -2.43 \pm 0.31$ kJ mol^{-1} for cellobiose; $K = 17.9 \pm 0.7$, $\Delta G^0 = -7.15 \pm 0.10$ kJ mol^{-1} and $\Delta H^0 = 2.26 \pm 0.48$ kJ mol^{-1} for gentiobiose; $K = 17.25 \pm 0.7$, $\Delta G^0 = -7.06 \pm 0.10$ kJ mol^{-1}, and $\Delta H^0 = 5.86 \pm 0.54$ kJ mol^{-1} for isomaltose; and $K \geq 513$, $\Delta G^0 \leq -15.5$ kJ mol^{-1}, and $\Delta H^0 = -4.02 \pm 0.15$ kJ mol^{-1} for maltose. The standard state is the hypothetical ideal solution of unit molality. Due to enzymatic inhibition by glucose, it was not possible to obtain reliable values for the equilibrium constants for the hydrolysis of either cellobiose or maltose. The entropy changes for the hydrolysis reactions are in the range 32 to 43 J mol^{-1} K^{-1}; the heat capacity changes are approximately equal to zero J mol^{-1} K^{-1}. Additional pathways for calculating thermodynamic parameters for these hydrolysis reactions are discussed.

The disaccharides cellobiose, gentiobiose, isomaltose, and maltose occur in living systems which use them both structurally and as energy sources. The glucosidic linkages in these four compounds are representative of the bonds occurring in starches and in cellulosic materials. These latter substances are among the most abundant organic materials occurring in nature. In addition to their fundamental chemical importance, there has been much study aimed at improved practical utilization of these substances.

Thermodynamic information on the hydrolysis reactions is very limited. In their reviews, Burton and co-workers (1, 2) and Wilhoit (3) based the Gibbs energy of formation of aqueous maltose upon a thermochemical calculation using enthalpies of combustion and solution, a third law entropy, and a solubility. The uncertainties of this type of calculation are inherently large. These reviews (1-3) contained no data on cellobiose, gentiobiose, or isomaltose. An alternative thermochemical pathway utilizes data obtained by Alexander (4) and by Fitting and Doudoroff (5). These workers used, respectively, the enzymes cellobiose phosphorylase and maltose phosphorylase for the phosphorylation of cellobiose and maltose to glucose 1-phosphate and glucose. The equilibrium constants which they determined can then be combined with equilibrium data for the hydrolysis of glucose 1-phosphate to glucose and inorganic phosphate to calculate equilibrium constants for the hydrolysis of cellobiose and maltose. More recently, equilibrium data on the hydrolysis of α-1,4 and α-1,6 linkages have been reported by van Beynum *et al.* (6). Takahashi *et al.* (7, 8) report calorimetric data on the hydrolysis of maltose and isomaltose. The aim of this research is to extend our knowledge of the thermodynamics of the hydrolysis of the disaccharides. To accomplish this we have used both high pressure liquid chromatography and microcalorimetry for the measurement of equilibrium constants and enthalpies of hydrolysis for the disaccharides cellobiose, gentiobiose, isomaltose, and maltose.

EXPERIMENTAL PROCEDURES

The sources of materials used in this study are as follows[1]: cellobiose, gentiobiose, isomaltose, and maltose were obtained from Sigma; the glucose was Standard Reference Material 917 from the National Bureau of Standards; the sodium acetate buffer was from J. T. Baker Chemical Co; the enzymes β-glucosidase (EC 3.2.1.21), isomaltase (EC 3.2.1.10), and maltase (EC 3.2.1.20) were from Sigma. The moisture contents of the substrates as determined by Karl Fischer titration were, in mass percent of water: cellobiose, 0.06; gentiobiose, 0.08; glucose, 0.03; isomaltose, 5.25, and maltose, 5.95. Corrections for these moisture contents were applied to both the calorimetric and equilibrium data. The substrates were found to be chromatographically pure using the chromatographic arrangement described below. The β-glucosidase was dialyzed against the buffer solution used for the equilibrium and calorimetric measurements to remove impurities which interfered with the chromatography. The isomaltase and maltase were in lyophilized forms and, when dissolved in buffer, they presented no interferences with the chromatographic measurements.

Measurements of the amounts of the substrates in solution were performed using a Dionex "BIOLC" equipped with an HPIC-AS6A anion exchange column and a pulsed amperometric detector. The mobile phase was aqueous sodium hydroxide (0.08-0.10 M). The flow rate was one ml/min. Typical retention times of the carbohydrates, in minutes, were: glucose, 5; cellobiose, 12; gentiobiose, 10; isomaltose, 8; and maltose, 19. The response factors of these substrates were determined daily.

Solutions of the substrates in buffer containing the appropriate enzyme were allowed to equilibrate with gentle stirring in a thermostatted water bath. Equilibrium was approached from two directions: starting with the disaccharide (the forward direction) and starting from glucose (the 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 2 days for both the gentiobiose and isomaltose hydrolyses. However, this was not the case for the experiments involving the hydrolysis of cellobiose and maltose. Equilibrations lasting for as

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[1] 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.

long as 14 days were attempted for these two systems. They were not successful. In all four equilibrium studies, significant systematic errors would have been incurred if the disaccharides had not been adequately separated during the chromatographic analysis.

The calorimeters are of the heat-conduction type and have calibration constants varying from 17 to 22 $W \cdot V^{-1}$. The sensitivity (units of $V \cdot W^{-1}$) is the inverse of the calibration constant. The sample vessels contain two compartments holding approximately 0.55 and 0.45 ml of solution, respectively. Complete descriptions of the calorimeters and their performance characteristics are given in Refs. 9 and 10. Measurements of reaction heat were performed by mixing, in the calorimeter, a substrate solution and an enzyme solution. The substrate solution was prepared by dissolving a known amount of the substrate (cellobiose, gentiobiose, isomaltose, or maltose) in a sodium acetate buffer solution. The enzyme solution was prepared by adding the same buffer solution to either the lyophilized or dialyzed enzyme. The extents of reaction for each of the disaccharides undergoing hydrolysis were determined by analyses of the reaction mixtures immediately following completion of the heat measurements. Typically, the calorimetric measurements extended over 1–2 h. The percentages of the disaccharides converted to glucose were found to be 97, 93, 92, and 98, respectively, for cellobiose, gentiobiose, isomaltose, and maltose at 298.15 K. At 310.15 K the percentages were 99, 93, and 99, respectively, for cellobiose, gentiobiose, and isomaltose. Eighty % of the maltose was converted to glucose at 304.65 K. The measured heat effects were corrected for incomplete reaction to obtain the molar enthalpies of reaction given later in this paper. It was found that a small amount of gentiobiose ($\leq$0.8% of the initial concentration of cellobiose) was formed during the cellobiose hydrolysis experiments. A correction was made for this effect using the enthalpy of hydrolysis of gentiobiose determined in this study.

The heat effects accompanying the mixing of the enzyme solution with the buffer averaged less than 0.22 mJ. Control experiments were also performed in which the enzyme solution was mixed with a solution prepared to have the composition of the reaction mixture at equilibrium. These control experiments served to determine if there was any heat effect associated with the interaction of the enzymes with the substrates which was independent of the heat effect associated with the chemical reaction. These control experiments yielded results which ranged from −1.7 to 3.7 mJ. These heat effects were applied as small corrections to the heats (60–300 mJ) measured for the hydrolysis reactions.

RESULTS AND DISCUSSION

The processes of primary interest in this investigation are the following:

$$\text{cellobiose(aq)} + H_2O\text{(liq)} = 2\ \text{glucose(aq)} \quad \text{(A)}$$

$$\text{gentiobiose(aq)} + H_2O\text{(liq)} = 2\ \text{glucose(aq)} \quad \text{(B)}$$

$$\text{isomaltose(aq)} + H_2O\text{(liq)} = 2\ \text{glucose(aq)} \quad \text{(C)}$$

$$\text{maltose(aq)} + H_2O\text{(liq)} = 2\ \text{glucose(aq)} \quad \text{(D)}$$

The thermodynamic equilibrium constants (K) for these hydrolysis reactions are given by:

$$K = \{[\text{glucose}]^2/(m^0\text{disaccharide}])\} \cdot \{\gamma^2\,(\text{glucose})/(a(H_2O)\gamma(\text{disaccharide}))\} \quad (1)$$

Here, the square brackets denote the molalities of either the glucose or the disaccharide in solution, γ is the activity coefficient of the indicated solute species, $a(H_2O)$ is the activity of the water, and m^0 is equal to 1 mol kg^{-1}. The standard state to which our measurements will be adjusted is, for the solute, the hypothetical ideal solution of unit molality and, for the water, the pure solvent. The reference temperature and pressure are 298.15 K and 0.1 MPa, respectively. The rigorous application of Equation 1 requires a knowledge of the activity coefficients of glucose and of the appropriate disaccharide in 0.1 M sodium acetate buffer at a pH of 5.65. The activity of water can be calculated from the activity coefficients of the solutes using the Gibbs-Duhem equation. In the absence of this information, we have used data (11, 12) on the activity coefficients of glucose and maltose in pure water and data on the interactions of sugars with electrolytes (13) to estimate a value of 1.006 for the second term in { } in Equation 1. Similarly, we have used enthalpies of dilution of glucose, maltose, and cellobiose (11, 14) in pure water to estimate a dilution correction of $\approx$0.10 kJ mol^{-1} which might be applied to our measured enthalpies to adjust them to the standard state. Since the inclusion of these corrections in the calculations causes effects of substantially less than the scatter in the experimental results and since they involve some estimated parameters, they will be dispensed with in the subsequent calculations.

The results of the equilibrium and calorimetric measurements are summarized in Tables I and II, respectively. The data are also shown in Figs. 1–3. Separate sets of experiments were performed starting with only disaccharide in solution (the forward direction) and with only glucose in solution (the reverse direction). It was possible to obtain agreement between equilibrium constants determined from the forward and reverse directions only for the experiments involving isomaltose and gentiobiose. Within the time frame of our measurements (up to 14 days), it was not possible to obtain agreement between the results from the forward and reverse directions in the experiments involving cellobiose and maltose. This is probably due to the combined effect of product inhibition of the enzymes maltase and β-glucosidase and the fact that the equilibrium constants for these two hydrolysis reactions are significantly larger than those for the hydrolysis of isomaltose and gentiobiose. On the basis of the experiments which were performed involving the hydrolysis of cellobiose and maltose, our findings were that the equilibrium constants for these respective hydrolyses were greater than or equal to 155 and 513 at 298.15 K. The corresponding inequalities for the Gibbs energy changes are $\Delta G^0 \leq -12.5$ kJ mol^{-1} for process

TABLE I

Equilibrium data for the hydrolysis of gentiobiose and isomaltose to aqueous glucose

The buffer was 0.1 M sodium acetate at pH 5.65. The enzymes β-glucosidase and isomaltase were used, respectively, for the gentiobiose and isomaltose hydrolysis experiments. Equilibrium was approached from two directions: starting with the disaccharide (the forward direction) and starting from glucose (the reverse direction). The initial concentrations of gentiobiose and isomaltose in the experiments from the forward direction were $\approx$15 mM. From the reverse direction, the initial concentration of glucose was $\approx$30 mM. The concentrations of β-glucosidase and isomaltase in these solutions were $\approx$5 g (kg solution)$^{-1}$. The equilibrium constants in columns two and three are the averages of three to five measurements. The equilibrium constants in column four are the averages of all of the data obtained at the specified temperatures. The uncertainties are 95% confidence limits.

	Hydrolysis of gentiobiose		
T/K	K (starting from gentiobiose)	K (starting from glucose)	K (average)
285.75	17.49 ± 0.52	16.92 ± 0.23	17.21 ± 0.29
292.25	17.63 ± 0.93	17.45 ± 0.71	17.53 ± 0.40
298.15	17.86 ± 0.42	17.56 ± 0.68	17.71 ± 0.32
304.15	18.11 ± 0.26	17.73 ± 1.2	17.92 ± 0.49
310.35	17.84 ± 0.93	19.21 ± 1.5	18.52 ± 0.82
316.15	19.53 ± 0.59	19.38 ± 0.47	19.45 ± 0.29
	Hydrolysis of isomaltose		
T/K	K (starting from isomaltose)	K (starting from glucose)	K (average)
286.35	15.88 ± 0.12	15.79 ± 0.22	15.83 ± 0.10
292.45	16.38 ± 0.44	16.01 ± 0.19	16.19 ± 0.22
298.15	17.26 ± 0.22	17.10 ± 0.21	17.18 ± 0.13
304.15	18.08 ± 0.38	17.97 ± 0.050	18.03 ± 0.16
310.25	18.83 ± 0.28	20.05 ± 0.20	19.44 ± 0.44
316.35	19.80 ± 0.27	19.58 ± 0.43	19.68 ± 0.20

TABLE II

Calorimetric data for the hydrolysis of cellobiose, gentiobiose, isomaltose, and maltose

The hydrolysis of isomaltose and maltose were carried out, respectively, using the enzymes isomaltase and maltase. The enzyme β-glucosidase was used for the hydrolysis of cellobiose and gentiobiose. The initial concentrations of the disaccharides were in the range 50–75 mM. The concentrations of the enzymes in these solutions were 10–25 g (kg solution)$^{-1}$. The buffer was 0.1 M sodium acetate at pH 5.65. These enthalpies have been corrected for incomplete conversion to glucose and, in the case of cellobiose hydrolysis, for the formation of a small amount of gentiobiose. Uncertainties refer to 95% confidence limits.

	Hydrolysis	
	T/K	ΔH/kJ mol^{-1}
Cellobiose	298.15	−2.37 ± 0.027
	304.55	−2.56 ± 0.046
	310.15	−2.34 ± 0.12
Gentiobiose	298.15	2.36 ± 0.40
	304.55	1.91 ± 0.16
	310.15	2.11 ± 0.090
Isomaltose	298.15	5.93 ± 0.059
	304.55	5.67 ± 0.11
	310.15	6.35 ± 0.048
	316.15	5.98 ± 0.16
Maltose	298.15	−4.02 ± 0.15
	304.65	−3.74 ± 0.14

A and $\Delta G^0 \leq -15.5$ kJ mol^{-1} for process D at 298.15 K.

The temperature dependence of the equilibrium constants (K) and the enthalpy changes (ΔH) are given by:

$$\Delta G^0_T = RT\ln K = \Delta H^0_\theta + \Delta C^0_p(T - \theta) + T(\Delta G^0_\theta - \Delta H^0_\theta)/\theta - T\Delta C^0_p \ln(T/\theta) \quad (2)$$

$$\Delta H^0_T = \Delta H^0_\theta + \Delta C^0_p(T - \theta) \quad (3)$$

In the above equations T is the thermodynamic temperature, θ is the reference temperature (298.15 K), and R is the gas constant (8.31451 J mol^{-1} K^{-1}). The heat capacity changes are assumed to be constant over the temperature range of interest.

Least squares fits of the enthalpy data in Table II to Equation 3 lead to $\Delta H^0 = -2.43 \pm 0.31$, 2.26 ± 0.48, 5.86 ± 0.54, and -4.02 ± 0.15 kJ mol^{-1} at 298.15 K, respectively, for processes A to D above. The corresponding values calculated for ΔC^0_p are 17 ± 38, -22 ± 60, 13 ± 48, and 43 J mol^{-1} K^{-1}. Since data for the hydrolysis of maltose were performed at only two different temperatures, a meaningful uncertainty could not be calculated for the heat capacity change accompanying its hydrolysis. The uncertainties given here and in the following discussion are, unless indicated otherwise, 95% confidence limits. None of these heat capacity changes differ significantly from zero. Therefore, we shall adopt a value of ΔC^0_p equal to zero J mol^{-1} K^{-1} over the temperature interval of our measurements (286–316 K) in all future calculations involving these processes.

Fitting the equilibrium data in Table I to Equation 2 with ΔC^0_p fixed at 0 J mol^{-1} K^{-1} leads to values of $\Delta G^0 = -7.147 \pm 0.047$ and $\Delta H^0 = 2.76 \pm 1.4$ kJ mol^{-1} for process B, the hydrolysis of gentiobiose. For process C, the hydrolysis of isomaltose, the calculated values are $\Delta G^0 = -7.062 \pm 0.049$ and $\Delta H^0 = 6.06 \pm 1.4$ kJ mol^{-1}. Thus, the values of the enthalpies determined from the temperature derivative of the equilibrium data are in good agreement with the enthalpies determined from the calorimetric measurements. We prefer the calorimetric results on the basis of their higher precision.

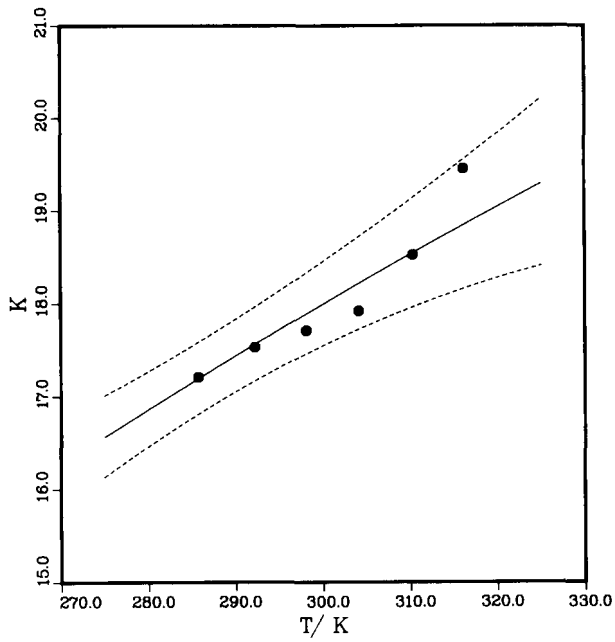

FIG. 1. **Equilibrium constants for the hydrolysis of gentiobiose to glucose as a function of temperature.** The experimental values are the solid octagons (●). The *solid line* was calculated using the following values for the hydrolysis reaction: $\Delta G^0 = -7.15 \pm 0.10$ kJ mol^{-1}, $\Delta H^0 = 2.26 \pm 0.48$ kJ mol^{-1}, and $\Delta C^0_p = 0$ J mol^{-1} K^{-1}. The *dashed lines* were obtained from the uncertainty intervals of these values.

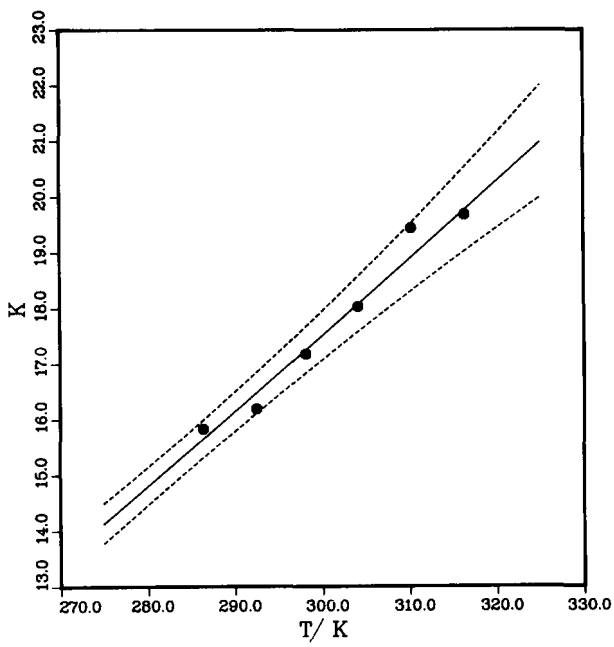

FIG. 2. **Equilibrium constants for the hydrolysis of isomaltose to glucose as a function of temperature.** The experimental values are the solid octagons (●). The *solid line* was calculated using the following values for the hydrolysis reaction: $\Delta G^0 = -7.06 \pm 0.10$ kJ mol^{-1}, $\Delta H^0 = 5.86 \pm 0.54$ kJ mol^{-1}, and $\Delta C^0_p = 0$ J mol^{-1} K^{-1}. The *dashed lines* were obtained from the uncertainty intervals of these values.

If the values of ΔH^0 in Equation 2 are fixed at those values determined from the calorimetric data, the calculated values of the Gibbs energy changes for processes B and C are -7.151 ± 0.043 and -7.064 ± 0.040 kJ mol^{-1}, respectively.

Table III contains the values of the thermodynamic parameters for the hydrolysis reactions of the disaccharides studied

herein. Since the uncertainties given in Table I did not include a component due to the uncertainties in the response factors, the final uncertainties assigned to both the equilibrium constants and the Gibbs energy changes have been increased appropriately. The best representations of the equilibrium constants for these hydrolysis reactions as a function of temperature (see Figs. 1 and 2) were calculated using the values of the thermodynamic parameters given in Table III. We now turn to a consideration of available data in the literature with which our results can be compared.

The only other direct equilibrium measurements in the literature are those of van Beynum *et al.* (6). They used high pressure liquid chromatography in an equilibrium study of the hydrolysis of α-1,4 and α-1,6 linkages. They state that the equilibrium constants for the hydrolysis of α-1,4 and α-1,6 linkages are independent of chain length and that they are equal to 194 and 33, respectively. They report essentially only their summary data and give few experimental details. We adjust their results to 298.15 K and obtain values 224 and 27, respectively, for the hydrolysis of maltose and isomaltose. Our results were $K \geq 513$ for the hydrolysis of maltose (α-1,4 linkage) and $K = 17.25 \pm 0.7$ for the hydrolysis of isomaltose (α-1,6 linkage) at 298.15 K. Van Beynum *et al.* (6) do not state what chromatographic procedures were used for the separation of the carbohydrates in their study. The anion exchange column and pulsed-amperometric detector which were used in this investigation provided separations of the disaccharides which cannot be obtained using cation exchange columns with a refractive index detector. Since the type of chromatographic arrangement used in this study was not available in 1981 to van Beynum *et al.* (6), a possible source of error in their measurements could be attributable to unsuspected chromatographic interferences and undetected side reactions.

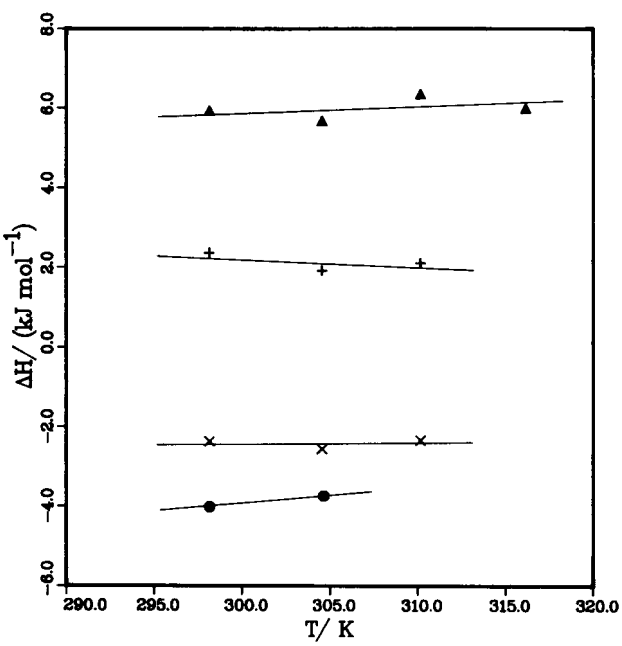

FIG. 3. **Enthalpies of hydrolysis of disaccharides to glucose as a function of temperature.** The disaccharides are cellobiose (×), gentiobiose (+), isomaltose (▲), and maltose (●). The *solid lines* are least squares fits to the data.

An alternative method of obtaining equilibrium constants for the hydrolysis of maltose and cellobiose uses the enzymes maltose phosphorylase and cellobiose phosphorylase, respectively, to catalyze the following reactions:

$$\text{maltose(aq)} + \text{HPO}_4^{2-}\text{(aq)} = \text{glucose(aq)} + \text{glucose 1-phosphate}^{-2}\text{(aq)} \quad \text{(E)}$$

$$\text{cellobiose(aq)} + \text{HPO}_4^{2-}\text{(aq)} = \text{glucose(aq)} + \text{glucose 1-phosphate}^{-2}\text{(aq)} \quad \text{(F)}$$

Equilibrium constants determined for these processes can then be combined with equilibrium data for the process:

$$\text{glucose 1-phosphate}^{-2}\text{(aq)} + \text{H}_2\text{O(liq)} = \text{glucose(aq)} + \text{HPO}_4^{2-}\text{(aq)} \quad \text{(G)}$$

Thus, the Gibbs energy changes for processes A and D can be calculated as $\Delta G^0_A = \Delta G^0_F + \Delta G^0_G$ and $\Delta G^0_D = \Delta G^0_E + \Delta G^0_G$, respectively. A Gibbs energy change of -17.97 kJ mol^{-1} for process G is calculated using the Gibbs energies of formation given in our recent review(11). Alexander (4) reported an equilibrium constant of 0.23 for the phosphorylation of cellobiose to glucose and to glucose 1-phosphate at 37 °C and at pH 7. Applying ionization, temperature, and ionic strength corrections (11, 16) to this result leads to $\Delta G^0 = 3.64$ kJ mol^{-1} for process F at 298.15 K. Combination with the value of ΔG^0 for process G leads to $\Delta G^0 = -14.3$ kJ mol^{-1} and $K = 320$ for process A at 298.15 K. Thus, our result for the hydrolysis of cellobiose ($K \geq 155$) is consistent with Alexander's (4) measurement and with the data leading to ΔG^0 for process G, the hydrolysis of glucose 1-phosphate. This pathway may provide the best currently available values for the equilibrium constants and Gibbs energy changes for processes A and F. Fitting and Doudoroff (5) report a value of 0.23 for the equilibrium constant for the phosphorylation of maltose at 37 °C and at pH 7. Applying corrections similar to those used on the cellobiose phosphorylation reaction, leads to a value of $\Delta G^0 = -14.3$ kJ mol^{-1} and $K = 320$ for process D, the hydrolysis of maltose, at 298.15 K. This result is inconsistent with our finding that the equilibrium constant for this process was greater than or equal to 513.

Takahashi *et al.* (7, 8) have performed calorimetric measurements on the hydrolysis of isomaltose and maltose. They report enthalpy changes of 5.44 ± 0.13 and -4.59 ± 0.028 kJ mol^{-1}, respectively, for the hydrolysis of isomaltose and maltose. Our results were 5.86 ± 0.54 and -4.02 ± 0.15 kJ mol^{-1}, respectively. Takahashi *et al.* (7, 8) did not analyze their final reaction mixtures and they assumed complete hydrolysis of

TABLE III

Thermodynamic parameters for the hydrolysis of disaccharides to glucose at 298.15 K

The heat capacity changes are approximately 0 J mol^{-1} K^{-1}. The standard state is the hypothetical ideal solution of unit molality.

Process	K	ΔG^0	ΔH^0	ΔS^0
		kJ mol^{-1}		*J mol*$^{-1}$ *K*$^{-1}$
Cellobiose(aq) + H_2O(liq) = 2 glucose(aq)	≥155	≤−12.5	−2.43 ± 0.31	≥33.8
Gentiobiose(aq) + H_2O(liq) = 2 glucose(aq)	17.89 ± 0.7	−7.15 ± 0.10	2.26 ± 0.48	31.6 ± 1.7
Isomaltose(aq) + H_2O(liq) = 2 glucose(aq)	17.25 ± 0.7	−7.06 ± 0.10	5.86 ± 0.54	43.3 ± 1.9
Maltose(aq) + H_2O(liq) = 2 glucose(aq)	≥513	≤−15.5	−4.02 ± 0.15	≥38.5

these two disaccharides. If we had done the same with our experimental data, the results would have been 5.47 and −3.94 kJ mol^{-1}, respectively. In any case, the results for the hydrolysis of isomaltose are clearly within the experimental uncertainties. The uncertainty assigned by Takahashi *et al.* (8) to the maltose hydrolysis result seems too small. We believe that a more realistic assignment of uncertainty to the maltose hydrolysis enthalpy determined by them (8) would show the two sets of results to be in accord.

Thermochemical cycle calculations using enthalpies of combustion and solution, third law entropies, solubilities, and activity coefficients can be used to obtain Gibbs energy and enthalpy changes for the hydrolysis reactions in aqueous solution. The accuracy of these thermochemical cycle calculations is highly dependent upon the accuracy of the enthalpies of combustion and/or third law entropies. Consequently, it requires exceptionally accurate data to approach the accuracy of either the Gibbs energy or enthalpy changes which are obtained from direct measurements on aqueous solutions. Since many of the values are based upon older measurements where proper sample characterization was not possible, the uncertainties in the calculated values are judged to be at least 5–10 kJ mol^{-1}. Thus, for maltose, we use the enthalpy of combustion selected by Domalski (17) in his review, a third law entropy from Anderson and Stegeman (18), the solubility (19) and activity coefficient (12), and a critically evaluated Gibbs energy of formation for aqueous glucose (11) to calculate $\Delta G^0 = -55.2$ kJ mol^{-1} for process D, the hydrolysis of aqueous maltose, at 298.15 K. While it is consistent with the result obtained in this study ($\Delta G^0 \leq -15.5$ kJ mol^{-1}) it is too negative by a substantial amount. Similar calculations on maltose monohydrate use data on enthalpies of combustion and solution (17, 20) and lead to a value of $\Delta H^0 = 1.6$ kJ mol^{-1} for the hydrolysis of aqueous maltose at 298.15 K. This is in approximate agreement with the direct measurement of -4.02 ± 0.15 kJ mol^{-1}. For cellobiose the reported (20, 21) enthalpies of solution differ by 4.2 kJ mol^{-1}. Using these two enthalpies of solution and the enthalpy of combustion given in Domalski's review (17), the enthalpy of hydrolysis of cellobiose is calculated to be between −17.8 and −22.0 kJ mol^{-1} at 298.15 K. The direct measurement yielded a value of -2.43 ± 0.31 kJ mol^{-1}. We believe that the difference is attributable primarily to errors in the enthalpy of combustion data.

Information on the heat capacity changes of the hydrolysis reactions can be obtained from direct heat capacity measurements on aqueous solutions. Jasra and Ahluwalia (20) report an apparent molar heat capacity (C_ϕ^0) of 635 J mol^{-1} K^{-1} for maltose(aq). Kawaizumi *et al.* (22) measured a value of C_ϕ^0 of 614 ± 20 J mol^{-1} K^{-1}. These results are respectively combined with a value of 336 J mol^{-1} K^{-1} for C_ϕ^0 for glucose(aq) from our recent review (11) and the heat capacity of water (23) to obtain values of ΔC_p^0 of −38 and −17 J mol^{-1} K^{-1} for process D, the hydrolysis of maltose. Jasra and Ahluwalia (20) also determined a value of C_ϕ^0 of 649 J mol^{-1} K^{-1} for cellobiose(aq). This leads to a value of ΔC_p^0 of −52 J mol^{-1} K^{-1} for process A, the hydrolysis of cellobiose. These calculated heat capacity changes may be more accurate than the results obtained from the temperature derivatives of the enthalpy changes measured in this study. While direct heat capacity measurements on aqueous solutions of gentiobiose and isomaltose would also be useful for extrapolating equilibrium constants and enthalpies to temperatures both higher and lower than those investigated, their use would have little effect on the calculated values of the Gibbs energy or enthalpy changes at 298.15 K obtained in this study.

Examination of the summary results in Table III shows that the enthalpy changes for the hydrolysis of cellobiose and maltose (1,4 linkages) are exothermic while the enthalpy changes for the hydrolysis of gentiobiose and isomaltose (1,6 linkages) are endothermic. Also, the absolute value of the enthalpy of hydrolysis of the cellobiose (β-1,4 linkage) is approximately half the absolute value of the enthalpy of hydrolysis of maltose (α-1,4 linkage). A similar rule is found to hold for the enthalpies of hydrolysis of gentiobiose and isomaltose which contain the corresponding β and α-1,6 linkages, respectively. Ono and Takahashi (24) have examined the available literature data on the hydrolysis of tri- and oligosaccharides. Based upon their own measurements (7, 25) on panose (one α-1,4 and one α-1,6 linkage), maltotriose (two α-1,4 linkages), and amylose ($\approx$6000 α-1,4 linkages) they present evidence that the enthalpy change corresponding to the hydrolysis of an individual glucosidic linkage is independent of the number of glucosidic linkages in a carbohydrate. Data on the Gibbs energy changes is more limited and is basically limited to the report of van Beynum *et al.* (6). They state that the equilibrium constants for the hydrolysis of α-1,4 and α-1,6 linkages are found to be constant in starches and dextrins having intermediate degrees of polymerization. It should be noted that the entropy changes (see Table III) for the hydrolysis of the disaccharides are reasonably close to each other. Any detailed explanation of these entropy differences would have to involve subtle structural effects.

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REFERENCES

1. Burton, K., and Krebs, H. A. (1953) *Biochem. J.* **54,** 94–107
2. Krebs, H. A., Kornberg, H. L., and Burton, K. (1957) *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **49,** 212
3. Wilhoit, R. C. (1969) in *Biochemical Microcalorimetry* (Brown, H. D., ed) pp. 33–81, 305–317, Academic Press, New York
4. Alexander, J. K. (1959) *Studies on a Purified Cellobiose Phosphorylase*, PhD. thesis, Montana State College
5. Fitting, C., and Doudoroff, M. (1952) *J. Biol. Chem.* **199,** 153–163
6. van Beynum, G. M. A., Roels, J. A., and van Tilberg, R. (1980) *Biotech. Bioeng.* **22,** 643–649
7. Takahashi, K., Yoshikawa, Y., Hiromi, K., and Ono, S. (1965) *J. Biochem. (Tokyo)* **58,** 251–254
8. Ono, S., Hiromi, K., and Takahashi, K. (1965) *J. Biochem. (Tokyo)* **57,** 799–807
9. Steckler, D. K., Goldberg, R. N., Tewari, Y. B., and Buckley, T. J. (1986) *J. Res. Nat. Bur. Stand.* **91,** 113–121
10. Steckler, D. K., Goldberg, R. N., Tewari, Y. B., and Buckley, T. J. (1986) *Computer Software for the Acquisition and Treatment of Calorimetric Data*, National Bureau of Standards Technical Note 1224, United States Government Printing Office, Washington, D. C.
11. Goldberg, R. N., and Tewari, Y. B. (1989) *J. Phys. Chem. Ref. Data*, in press
12. Miyajima, K., Sawada, M., and Nagaki, M. (1983) *Bull. Chem. Soc. Jpn.* **56,** 1954–1957
13. Morel, J. P., Lhermet, C., and Morel-Desrosiers, N. (1988) *J. Chem. Soc. Faraday Trans. 1* **84,** 2567–2571
14. Gaffney, S. H., Haslam, E., Lilley, T. H., and Ward, T. R. (1988) *J. Chem. Soc. Faraday Trans. 1* **84,** 2545–2552
15. Gucker, F. T., Jr., Pickard, H. B., and Planck, R. W. (1939) *J. Am. Chem. Soc.* **61,** 459 (1939)
16. Tewari, Y. B., Steckler, D. K., Goldberg, R. N. and Gitomer, W. L. (1988) *J. Biol. Chem.*, **263,** 3670–3675
17. Domalski, E. S. (1972) *J. Phys. Chem. Ref. Data* **1,** 221–277
18. Anderson, A. G., and Stegeman, G. (1941) *J. Am. Chem. Soc.* **63,** 2119–2121
19. Stephen, H., and Stephen, T. (1963) *Solubilities of Inorganic and*

Organic Compounds, Vol. 1, Part 1, pp. 517–518, Pergamon Press, New York
20. Yasra, R. V., and Ahluwalia, J. C. (1984) *J. Chem. Thermodyn.* **16,** 583–590
21. Taylor, J. B. (1957) *Trans. Faraday Soc.* **53,** 1198–1203
22. Kawaizumi, F., Nishio, N., Nomura, H., and Miyahara, Y. (1981) *J. Chem. Thermodyn.* **13,** 89–98
23. Wagman, D. D., Evans, W. H., Parker, V. B., Schumm, R. H., Halow, I., Bailey, S. M., Churney, K. L., and Nuttall, R. L. (1982) *J. Phys. Chem. Ref. Data* **11,** Suppl. 2, 1–392
24. Ono, S., and Takahashi, K. (1969) in *Biochemical Microcalorimetry* (Brown, H. D., ed), pp. 99–106, Academic Press, New York
25. Takahashi, K., Hiromi, K., and Ono, S. (1965) *J. Biochem.* (*Tokyo*) **58,** 255–258