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Thermodynamics of hydrolysis of oligosaccharides

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Microcalorimetry has been used to determine enthalpy changes for the hydrolysis of a series of oligosaccharides. High-pressure liquid chromatography was used to determine the extents of reaction and to check for any possible side reactions. The enzyme glucan 1,4-α-glucosidase was used to bring about the following hydrolysis reactions: (A) maltose(aq) + H_2O(liq) = 2D-glucose(aq); (B) maltotriose(aq) + 2H_2O(liq) = 3D-glucose(aq); (C) maltotetraose(aq) + 3H_2O(liq) = 4D-glucose(aq); (D) maltopentaose(aq) + 4H_2O(liq) = 5D-glucose(aq); (E) maltohexaose(aq) + 5H_2O(liq) = 6D-glucose(aq); (F) maltoheptaose(aq) + 6H_2O(liq) = 7D-glucose(aq); (G) amylose(aq) + $n$$H_2O$(liq) = ($n$ + 1) D-glucose(aq); and (H) panose(aq) + 2H_2O(liq) = 3D-glucose(aq); (J) isomaltotriose(aq) + 2H_2O(liq) = 3D-glucose(aq). The enzyme β-fructofuranosidase was used for the reactions: (K) raffinose(aq) + H_2O(liq) = α-D-melibiose(aq) + D-fructose(aq); and (L) stachyose(aq) + H_2O(liq) = o-α-D-galactopyranosyl-(1 → 6)-α-o-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose + D-fructose(aq). The results of the calorimetric measurements (298.15 K, 0.1 M sodium acetate buffer, pH 4.44–6.00) are: $\Delta H^\circ_A = -4.55 \pm 0.10$, $\Delta H^\circ_B = -9.03 \pm 0.10$, $\Delta H^\circ_C = -13.79 \pm 0.15$, $\Delta H^\circ_D = -18.12 \pm 0.10$, $\Delta H^\circ_E = -22.40 \pm 0.15$, $\Delta H^\circ_F = -26.81 \pm 0.20$, $\Delta H^\circ_H = 1.46 \pm 0.40$, $\Delta H^\circ_J = 11.4 \pm 2.0$, $\Delta H^\circ_K = -15.25 \pm 0.20$, and $\Delta H^\circ_L = -14.93 \pm 0.20$ kJ mol^{-1}. The enthalpies of hydrolysis of two different samples of amylose were 1062 ± 20 and 2719 ± 100 kJ mol^{-1}, respectively. These processes correspond to the hydrolysis of the following linkages: glucose-glucose (α,1 → 4) glucose-glucose (α,1 → 6), and glucose-fructose (1 → 6). The respective enthalpy changes accompanying the hydrolysis of these linkages are −4.53, 5.8 and −15.0 kJ mol^{-1}. Both these results and available thermodynamic data in the literature demonstrate that additivity works well in predicting the thermodynamics of hydrolysis reactions involving oligosaccharides.

1. Introduction

Earlier work by Takahashi et al. [1,2] indicated that additivity schemes were useful for the prediction of the enthalpy changes accompanying the hydrolysis of carbohydrates containing α-1,4 linkages. Their results [1,2] seemed particularly important because of the possibility that thermodynamic information on the hydrolysis of disaccharides containing various types of linkages could provide the basis for the estimation of the thermodynamics of processes involving oligo- and polysaccharides. Thus, the available thermodynamic data on the hydrolysis of disaccharides [3–6] could prove to be extremely useful in the estimation of thermodynamic data for the vast multiplicity of carbohydrates formed as possible permutations and combinations of the available monosaccharides and their interconnecting linkages. Towards this end we have (a) repeated some of the measurements of Takahashi et al. [1,2] on compounds containing α-1,4 and α-1,6 linkages, (b) extended their measurements on substances containing α-1,4 linkages to include the compounds

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maltotetraose, maltopentaose, maltohexaose, and maltoheptaose, and (c) hydrolyzed the oligosaccharides isomaltotriose, raffinose, and stachyose [1]. The determination of the enthalpy changes accompanying the hydrolysis of these oligosaccharides makes it possible to quantitatively test the extent to which additivity schemes work on carbohydrates. We have also examined the available information in the literature which bears upon the generalization of additivity estimation schemes to include Gibbs energy and entropy data for carbohydrates containing various types of linkages.

2. Experimental

The materials used in this study were obtained [2] from Sigma with only a few exceptions. The α-D-glucose was obtained from the National Institute of Standards and Technology, the D-fructose and D-galactose were from Pfanstiehl, and the sodium acetate was from J.T. Baker. The carbohydrates used in this investigation and their moisture contents in mass percent, as determined in this laboratory by Karl Fischer titration, are: maltose, 6.0; maltotriose, 3.9; maltotetraose, 5.6; maltopentaose, 3.9; maltohexaose, 5.4; maltopheptaose, 4.8; panose, 0.69; isomaltotriose, 4.9; amylose (sample no. 1), 16.3; amylose (sample no. 2), 9.4; raffinose, 16.2; and stachyose, 10.3. These moisture contents were applied as corrections to the calorimetric measurements. The determination of the moisture contents in the amylose samples was difficult due to the very slow (≈ 1 h) rate of solubilization of these samples in the formamide and Karl Fischer reagent. Thus, while the uncertainties in the moisture contents of the other substances are in the range 5–10% of the values given above, the uncertainties in the moisture contents of the amylose samples are estimated to be about 20% of the reported values. The oligosaccharides were found to be pure using the chromatographic procedures described below.

Characterization of the two amylose samples, both of which originated from potato starch, was performed using a Spectrophysics HPLC with a refractive index detector. A Phenomenex Linear GPG Column (5 μm bead size) thermostatted at 60 °C was used with a mobile phase consisting of 50% DMSO and 50% acetonitrile. Calibration was done using pullulan standards obtained from Polymer laboratories. Amylose sample no. 1 had a number average molecular weight [3] of 45 400 and a mass average molecular weight of 99 900. Sample no. 2 was found to have a number average molecular weight of 135 000 and a mass average molecular weight of 7 746 000. The uncertainties in these molecular weights are estimated to be 8–10% of the stated values.

The enzymes used in this study are glucan 1,4-α-glucosidase (EC 3.2.1.3) and β-fructo-

[1] Systematic names for the saccharides considered in this study are: isomaltotriose, *o*-α-D-glucopyranosyl-(1 → 6)-*o*-α-D-glucopyranosyl-(1 → 6)-D-glucopyranose; panose, *o*-α-D-glucopyranosyl-(1 → 6)-*o*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose; raffinose, *o*-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl β-D-fructofuranoside; and stachyose, *o*-α-D-galactopyranosyl-(1 → 6)-*o*-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl β-D-fructofuranoside; and α-D-melibiose, 6-*o*-α-D-galactopyranosyl-D-glucopyranose. Maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose are [*o*-α-D-glucopyranosyl-(1 → 4)-]$_n$ α-D-glucopyranose, where $n = 1$, 2, 3, 4, 5, and 6, respectively; amylose is a mixture of polysaccharides of this type where n is a large number. The compound *o*-α-D-galactopyranosyl-(1 → 6)-*o*-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose has no common name. Laminaribiose, laminaritriose, and laminaritetraose are [*o*-β-D-glucopyranosyl-(1 → 3)-]$_n$ D-glucopyranose, where $n = 1$, 2 and 3, respectively. Cellobiose and cellotriose are [*o*-β-D-glucopyranosyl-(1 → 4)-]^{1}n β-D-glucopyranose, where $n = 1$ and 2, respectively. Aqueous inorganic orthophosphate is abbreviated as P_i.

[2] 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.

[3] The number average molecular weight is equal to $\Sigma N_i M_i/(\Sigma N_i)$ where N_i is the number of molecules having a molecular weight M_i. The mass average molecular weight is equal to $\Sigma m_k M_i/(\Sigma m_i)$ where the m_i are the masses of material in the different molecular weight fractions [15].

[4] Glucan 1,4-α-glucosidase is commonly called glucoamylase or amyloglucosidase. The enzyme β-fructofuranosidase is commonly called invertase.

furanosidase (EC 3.2.1.26) [4]. The glucan 1,4-α-glucosidase was used for the hydrolysis of the following compounds: maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, panose, isomaltotriose, and the two different samples of amylose. The β-fructofuranosidase was used for the hydrolysis of raffinose and stachyose. Both enzymes were in lyophilized form.

The calorimetric and chromatographic procedures are similar to those used previously [3,6]. Chromatographic analyses of the reaction mixtures were performed immediately following the calorimetric measurements. These analyses served to determine the extents of reaction, to check for any possible side reactions, and to confirm the stoichiometry of the reactions. For example, following the hydrolysis reactions of raffinose and stachyose using β-fructofuranosidase the chromatograms showed no evidence of side reactions. Mass balance checks confirmed, within the combined experimental errors of the chromatographic analyses and the moisture determinations, the stoichiometry of all of the processes studied herein. Analyses of the reaction mixtures following the hydrolysis of panose showed the presence of isomaltose (0.75%), unreacted panose (0.03%), and glucose (99.22%). Similar analyses following the hydrolysis of isomaltotriose showed isomaltose (9.8%), unreacted isomaltotriose (48.7%), and glucose (41.5%). The values in parentheses given here are average mole percentages of these substances found on analysis of the reaction mixtures. Appropriate corrections were applied for the amounts of unreacted substrate and for the presence of the isomaltose in solution. In making the latter correction, the enthalpy of hydrolysis of isomaltose to glucose was taken from our earlier work [3]. Analysis of the reaction mixtures formed on hydrolysis of the series of maltose related compounds (see table 1) showed the presence of other disaccharides in amounts less than 0.8 mol%. Since these disaccharides could not be identified, it was not possible to make any correction due to their presence. In the calorimetric measurements, 'blank' heats of −0.14 and −0.79 mJ were determined for the enzymes glucan 1,4-α-glucosidase (EC 3.2.1.3) and β-fructofuranosidase (EC 3.2.1.26), respectively. All of the hydrolysis reactions were rapid and 'complete' within approx. 30 min with the exception of the hydrolysis of isomaltotriose.

Table 1

Enthalpies of hydrolysis of carbohydrates containing α-1,4 linkages

All reactions were carried out using glucan 1,4-α-glucosidase ($\approx 4\ g\ l^{-1}$) in sodium acetate buffer (0.1 M and pH 4.44). The concentrations of the substrates in solution were: 0.013–0.016 M for the maltose and maltotriose, 0.008–0.010 M for the maltotetraose and maltopentaose, and 0.005–0.009 M for the maltohexaose and maltoheptaose. The averages are the results of five to seven measurements. The uncertainties are 95% confidence limits. The enthalpy change in the last column was obtained by dividing the enthalpy change in the third column by the number of α-1,4 linkages in a molecule of the oligosaccharide hydrolyzed.

Carbohydrate	T (K)	$-\Delta H$ (kJ mol^{-1})	$-\Delta H$ (kJ linkage^{-1})
Maltose	298.15	4.55±0.04	4.55
Maltotriose	298.15	9.03±0.03	4.52
Maltotetraose	298.15	13.79±0.09	4.60
Maltopentaose	298.15	18.12±0.05	4.53
Maltohexaose	298.15	22.40±0.10	4.48
Maltohexaose	304.65	22.63±0.10	4.53
Maltohexaose	311.15	22.16±0.29	4.43
Maltoheptaose	298.15	26.81±0.14	4.47
Maltoheptaose	304.65	27.14±0.29	4.52
Maltoheptaose	311.15	26.38±0.29	4.40

Attempts were made to determine equilibrium constants for the various hydrolysis processes studied herein. These attempts were not successful due to the very large extents of reaction for these reactions.

Since the amylose samples were insoluble in the buffer solutions used for the oligosaccharides, it was necessary to use a solubilization procedure similar to that used by Takahashi et al., [1]. Absolute ethanol (1.24 g) and distilled water (20.0 g) were added to an amylose sample (0.10 g). This solution was then mixed using a magnetic stirrer and concentrated sodium hydroxide (0.74 g, ≈ 20 M) was added slowly. Glacial acetic acid (0.79 g, 17.4 M) was then added to bring the pH to 5.31. This solution is designated as the 'amylose solution'. In doing this, the amounts of solution added were carefully weighed so as to be able to prepare subsequently a nearly identical solution contain-

ing all of the aforementioned reagents but without any amylose. This latter solution is designated as the 'synthetic buffer solution'. This solution in turn was used to prepare an 'enzyme solution' containing the glucan 1,4-α-glucosidase. A weighed amount of this enzyme solution was placed in one side of the calorimeter vessel and a weighed portion of the amylose solution was placed in the other side of the calorimeter vessel. Following equilibration in the microcalorimeter the two solutions were mixed and the heat was measured. The purpose of having two nearly identical solutions was to be able to minimize the heat that would be produced by having an imbalance in the pH and/or ionic strengths of the two solutions. That this was accomplished was demonstrated by measuring the heat produced by mixing the synthetic buffer solution with the amylose solution. The heat measured in these control experiments was $-(0.63 \pm 1.1)$ mJ, while the reaction heats were in the range 30–50 mJ. The heat measured in these control experiments does not differ significantly from the blank heat (-0.14 mJ) measured for the glucan 1,4-α-glucosidase solution. Since these experiments were performed using a much more concentrated sodium acetate buffer solution ($\approx$ 0.57 M) containing ethanol, an additional control experiment was also performed in which maltopentaose was used in the synthetic buffer solution instead of amylose. Thus, a fairer comparison could be made between the enthalpy changes measured for the hydrolysis of the α-1,4 linkages in both the maltopentaose and the amylose samples.

3. Results and discussion

The processes which have been investigated herein are:

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

$$\text{maltotriose(aq)} + 2H_2O\text{(liq)} = 3\text{D-glucose(aq)} \quad \text{(B)}$$

$$\text{maltotetraose(aq)} + 3H_2O\text{(liq)} = 4\text{D-glucose(aq)} \quad \text{(C)}$$

$$\text{maltopentaose(aq)} + 4H_2O\text{(liq)} = 5\text{D-glucose(aq)} \quad \text{(D)}$$

$$\text{maltohexaose(aq)} + 5\ H_2O\text{(liq)} = 6\text{D-glucose(aq)} \quad \text{(E)}$$

$$\text{maltoheptaose(aq)} + 6H_2O\text{(liq)} = 7\text{D-glucose(aq)} \quad \text{(F)}$$

$$\text{amylose(aq)} + nH_2O\text{(liq)} = (n+1)\ \text{D-glucose(aq)} \quad \text{(G)}$$

$$\text{panose(aq)} + 2H_2O\text{(liq)} = 3\text{D-glucose(aq)} \quad \text{(H)}$$

$$\text{isomaltotriose(aq)} + 2H_2O\text{(liq)} = 3\text{D-glucose(aq)} \quad \text{(J)}$$

$$\text{raffinose(aq)} + H_2O\text{(liq)} = \text{α-D-melibiose(aq)} + \text{D-fructose(aq)} \quad \text{(K)}$$

$$\text{stachyose(aq)} + H_2O\text{(liq)} = \text{o-α-D-galactopyranosyl-(1 → 6)-o-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose} \times\text{(aq)} + \text{D-fructose(aq)} \quad \text{(L)}$$

Results of the calorimetric measurements for the hydrolysis of the oligosaccharides are summarized in tables 1 and 2. Based upon these results the enthalpy changes for the above processes are: $\Delta H^\circ_A = -4.55 \pm 0.10$, $\Delta H^\circ_B = -9.03 \pm 0.10$, $\Delta H^\circ_C = -13.79 \pm 0.15$, $\Delta H^\circ_D = -18.12 \pm 0.10$, $\Delta H^\circ_E = -22.40 \pm 0.15$, $\Delta H^\circ_F = -26.81 \pm 0.20$, $\Delta H^\circ_H = 1.46 \pm 0.40$, $\Delta H^\circ_J = 11.4 \pm 2.0$, $\Delta H^\circ_K = -15.25 \pm 0.20$, and $\Delta H^\circ_L = -14.93 \pm 0.20$ kJ

Table 2

Enthalpies of hydrolysis of some oligosaccharides at 298.15 K

The enzyme β-fructofuranosidase (≈ 3 g l^{-1}) was used for the hydrolysis of raffinose and stachyose. The buffer was 0.10 M sodium acetate at pH 6.00. The concentrations of the raffinose and stachyose were in the range 0.0077–0.0081 M. Glucan 1,4-α-glucosidase (≈ 5 g l^{-1}) was used for the hydrolysis of the panose and isomaltotriose which were present in the concentration range 0.011–0.013 M. Sodium acetate buffer (0.10 M and pH 4.44) was used for the hydrolysis experiments involving the panose and isomaltotriose. The averages are the results of five or six measurements. The uncertainties are 95% confidence limits.

Carbohydrate	ΔH (kJ mol^{-1})
Panose	1.46 ± 0.34
Isomaltotriose	11.4 ± 1.6
Raffinose	-15.25 ± 0.11
Stachyose	-14.93 ± 0.09

mol^{-1} at 298. 15 K. The enthalpies of hydrolysis of two different samples of amylose (process G) are 1062 ± 20 and 2719 ± 100 kJ mol^{-1}, respectively. The uncertainties assigned to the enthalpy changes have been increased above the 95% confidence limits. This was done for the same reasons as in the previous study [6], namely, the possibility of systematic errors attributable to sample purity, in the determination of the extent of reaction,possible side reactions, and the adjustment to the standard state. Measurements at temperatures other than 298.15 K were performed for both maltohexaose and maltoheptaose. Least-squares fits to this data lead to heat capacity changes (ΔC_p^o) of -18 and -33 J mol^{-1} K^{-1} for processes (E) and (F), respectively. The standard deviations for these fits are 31 and 48 J mol^{-1} K^{-1}, respectively. Thus, the values for these heat capacity changes must be considered approximate.

The results in the last column in table 1 were obtained by dividing the molar enthalpy change by the number of α-1,4 linkages in a molecule of the oligosaccharide hydrolyzed. The results are essentially constant and they are all consistent with an average value of -4.53 ± 0.04 kJ $linkage^{-1}$. Thus, a rule of additivity is found to work remarkably well for this series of compounds containing only α-1,4 linkages. The issue of whether additivity worked was also put to a test using data for both isomaltotriose and panose.

The enthalpy of hydrolysis of isomaltotriose, which contains two α-1,6 linkages, was found to be 11.4 ± 2.0 kJ mol^{-1}. Use of the additivity rule and the enthalpy of hydrolysis of isomaltose of 5.86 ± 0.54 kJ mol^{-1} [3] leads to a predicted value of 11.7 kJ mol^{-1} for the hydrolysis of isomaltotriose. For panose, which contains one α-1,4 and one α-1,6 linkage, the measured enthalpy of hydrolysis was 1.46 ± 0.40 kJ mol^{-1}. Using the enthalpy of hydrolysis of isomaltose and the enthalpy of hydrolysis of maltose determined herein, leads to a predicted value of 1.31 kJ mol^{-1}. Thus, again, the additivity rule was found to hold.

The enthalpies of hydrolysis of raffinose and stachyose were found to be -15.25 ± 0.20 and -14.93 ± 0.20 kJ mol^{-1}, respectively, at 298.15 K. The enthalpy of hydrolysis of sucrose, which involves the breaking of the same type of bond as in the hydrolysis of raffinose and stachyose, is -14.93 ± 0.16 kJ mol^{-1}. These three values are very close to being equal and the results are consistent with the rule that non-bonded interactions can be neglected in predicting the thermodynamics of these hydrolysis reactions. This rule is also consistent with the rule of additivity.

TABLE 3

Enthalpies of hydrolysis of amylose and maltopentaose in concentrated sodium acetate buffer at 298.15 K

The hydrolysis reactions were carried out using glucan 1,4-α-glucosidase (≈ 3 g l^{-1}) in sodium acetate buffer (0.57 M and pH 5.31) containing ethanol. The concentration of the maltopentaose was 0.0037–0.0043 M. The concentrations of the two amylose samples in the final reaction mixture were approx. 2 g l^{-1}. The number average molecular weights were used to calculate the enthalpy changes in kJ mol^{-1}. The average number of linkages was taken to be the number average molecular weight divided by the molecular weight of a glucosyl unit (162.13). Thus, amylose samples no. 1 and 2 had 280 and 833 linkages, respectively. Six experiments were performed on each sample. The uncertainties are 95% confidence limits.

Carbohydrate	$-\Delta H$ (kJ mol^{-1})	$-\Delta H$ (kJ $linkage^{-1}$)
Maltopentaose	15.17 ± 0.23	3.79 ± 0.06
Amylose (sample no. 1)	1062 ± 9	3.79 ± 0.03
Amylose (sample no. 2)	2719 ± 58	3.26 ± 0.07

A test of the issue of whether this additivity rule extended to polysaccharides was addressed by the experiments performed on the amylose samples, the results of which are given in table 3. Here the results were obtained using the solubilization procedure described in section 2. Thus, the measurements were performed under substantially different conditions than those used to obtain in the data in tables 1 and 2. The findings in table 3 are that for one amylose sample the enthalpy change in kJ per linkage broken is the same as in the maltopentaose sample hydrolyzed under similar conditions, while it is different for the second sample. A possible explanation for this difference, other than a weakening of the additivity rule, may be a lack of homogeneity in this amylose sample due to the presence of other types of linkages, including cross-linking. The generalization of all of these results in terms of an additivity estimation method and neglect of non-bonded interac-

tions will be done following a summary of relevant data from the literature.

Jasra and Ahluwalia [7] have determined apparent molar heat capacities for aqueous solutions of raffinose, stachyose, and α-D-melibiose. The results on raffinose and α-D-melibiose can be used in conjunction with the apparent molar heat capacity of D-fructose [8] to calculate a heat capacity change of 16 J mol^{-1} for process (K), the hydrolysis of raffinose to α-D-melibiose and D-fructose. This value is consistent with the small heat capacity changes found on the hydrolysis of disaccharides [6]. The apparent molar heat capacity of stachyose cannot be used in such a calculation in the absence of heat capacity data for the compound *O*-α-D-galactopyranosyl-(1 → 6)-*o*-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose.

Calorimetric data have been reported for the hydrolysis of maltose [3,10], maltotriose [1], amylose [1], and panose [9]. For the hydrolysis of maltose the earlier results [3,10] were $\Delta H^{\circ} = -4.02$ and -4.59 kJ mol^{-1} at 298.15 K, respectively. Thus, the result of -4.55 ± 0.10 kJ mol^{-1} obtained herein is in excellent agreement with the result of Ono et al. [10] but differs with the result obtained in our earlier study [3] by 0.5 kJ mol^{-1}, an amount that is just outside the range of the assigned uncertainties. In the earlier study [3], where the enzyme α-glucosidase (EC 3.2.1.20) was used, the rate of reaction was very slow, i.e., the calorimetric experiments lasted 90–120 min, and the extent of reaction was not complete. In the current study, however, the reaction was both rapid and essentially complete. On this basis the current result is to be preferred over our previously reported one. The result of -8.83 ± 0.13 kJ mol^{-1} for the hydrolysis of maltotriose obtained by Takahashi et al. [1] differs by only 0.2 kJ mol^{-1} from that of -9.03 ± 0.10 kJ mol^{-1} obtained herein. The two results are considered to be in agreement. Takahashi et al. [9] report a result of 0.586 ± 0.013 kJ mol^{-1} for the hydrolysis of panose. In obtaining this result, Takahashi et al. [9] applied a mutarotation correction of 837 J mol^{-1}. This correction should not be needed since the hydrolysis reaction takes the glucose to the equilibrium amounts of α and β forms under these conditions. If this correction is not made, the result of Takahashi et al. [9] becomes 1.42 ± 0.013 kJ mol^{-1}. While their assigned uncertainty seems unusually small, the result is in excellent agreement with the enthalpy change of 1.46 ± 0.40 kJ mol^{-1} obtained in this study. Takahashi et al. [1] also studied the hydrolysis of amylose and used a solubilization procedure like the one used herein. However, they were able to achieve solubilization of their amylose sample using a lower concentration of sodium hydroxide. The result they obtained for the enthalpy of hydrolysis of an α-1,4 glucosidic linkage in that amylose sample was -4.31 kJ $linkage^{-1}$. This result was close to that which they obtained [1] for the hydrolysis of this linkage in a sample of maltotriose, namely, -4.41 kJ $linkage^{-1}$.

Enthalpies of combustion have been determined for the crystalline forms of raffinose and stachyose [11]. However, in the absence of enthalpies of solution, these data cannot be used in the thermochemical cycle calculations to obtain enthalpies of hydrolysis of these sugars. There are no heat capacities on the solid forms of these saccharides and consequently no third law entropies. Also, surprisingly, there are no solubilities for any of these substances except maltose. Thus, with the exception of maltose, it is not possible to make any comparisons of the enthalpies or Gibbs energies of hydrolysis of any of these saccharides with data obtained on their crystalline forms. This comparison was made in our earlier study [3].

It was not possible to determine equilibrium constants for any of these hydrolysis reactions due to the very large extent to which these reactions go to completion in the direction of hydrolysis. Also, none have been reported in the literature. Nevertheless, it is desirable to determine if the equilibrium constants for similar hydrolysis reactions change with increasing numbers of glucosidic linkages in similar carbohydrates. Thus, Goldemberg et al. [12] studied the phosphorylation reactions of laminaribiose, laminaritriose, and laminaritetraose:

$$\text{laminaribiose(aq)} + P_i = \text{D-glucose(aq)} + \alpha\text{-D-glucose 1-phosphate(aq)} \quad \text{(M)}$$

laminaritriose(aq) + P_i

= laminaribiose(aq)

+ α-D-glucose 1-phosphate(aq) (N)

laminaritetraose(aq) + P_i

= laminaritriose(aq)

+ α-D-glucose 1-phosphate(aq) (O)

The equilibrium constants they report [12] for the above three reactions (310.15 K, 0.04 M imidazole buffer, and pH 6.5) are 0.30, 0.26, and 0.36, respectively. These equilibrium constants are the same within their experimental error. Similarly, equilibrium constants have been reported [13,14] for the reactions:

cellobiose(aq) + P_i

= D-glucose(aq) + α-D-glucose 1-phosphate(aq) (P)

cellotriose(aq) + P_i

= cellobiose(aq) + α-D-glucose 1-phosphate(aq) (Q)

Alexander [13] reported an equilibrium constant (310.15 K, 0.01 M barbital acetate buffer at pH 7.0) of 0.23 for process (P). Later, Sheth and Alexander [14] reported a value of 0.4 for the equilibrium constant (310.15 K, 0.01 M Tris buffer at pH 7.5) for reaction (Q). These values are approximate and can also be considered to be equal within experimental error and neglecting the application of ionization corrections to adjust the values to a common pH. Equilibrium constants for hydrolysis reactions of these saccharides can be calculated using the Gibbs energy change for the phosphorylation reaction and the Gibbs energy change for the process:

α-D-glucose 1-phosphate(aq) + H_2O(liq)

= D-glucose(aq) + P_i (R)

Thus, for example, the Gibbs energy change for the hydrolysis of laminaritriose to laminaribiose is equal to $\{\Delta G^\circ_N + \Delta G^\circ_R\}$. Since the Gibbs energy changes for these phosphorylation changes are equal, the Gibbs energy changes for the hydrolysis reactions within a given saccharide series will also be equal.

Based upon the above experimental evidence the generalization can be made that additivity will work in predicting Gibbs energy and enthalpy changes. Thus, non-bonded interactions can be neglected. Clearly, additivity must then also hold for entropy changes and most likely for heat capacity changes. Therefore, the molar Gibbs energy, enthalpy, entropy, and heat capacity changes, i.e., the thermodynamic changes, for the process:

$(sacch)_n$(aq) + H_2O(liq)

= sacch(aq) + $(sacch)_{n-1}$(aq) (S)

will be equal to the corresponding changes for the hydrolysis of the disaccharide containing the same linkage broken in the above process Here, 'sacch' is used as an abbreviation for any monosaccharide. This generalization regarding the enthalpy has been made previously by Takahashi et al. [1,2]. While this rule is supported to within a few tenths of a kilojoule per mole by a reasonable body of data involving enthalpies of reaction, additional Gibbs energy and heat capacity data would be useful in determining just how precisely the rule holds for these latter quantities. This rule also has implications for the thermodynamics of the homologizing reaction:

$$(sacch)_m + (sacch)_n = (sacch)_{m-1} + (sacch)_{n+1} \quad (T)$$

Namely, the Gibbs energy, enthalpy, entropy, and heat capacity changes for this process are equal to zero. While the latter rule is implied by the additivity rule, the converse does not apply. It should also be noted that all the available thermodynamic data indicate the very large extent to which the hydrolysis reactions involving saccharides go to completion in the direction of hydrolysis. This is desirable if it is the breakdown of saccharides which is sought. However, it also implies that any efficient synthetic pathways leading to oligo- or polysaccharides must proceed via different types of reactions.

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