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Thermodynamics of the hydrolysis and cyclization reactions of α-, β-, and γ-cyclodextrin

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Abstract

A thermodynamic investigation of the hydrolysis and cyclization reactions of cyclomalto-hexa-, hepta-, and octa-ose (α-, β-, and γ-cyclodextrins) has been performed using microcalorimetry and high-performance liquid-chromatography. The calorimetric measurements lead to standard molar enthalpy changes $\Delta_r H_m^\circ$ ($T = 298.15$ K, KH_2PO_4 buffer ($m = 0.10$ mol kg^{-1}), pH = 4.58 to 5.15) for the following reactions:

α-cyclodextrin(aq) + $6\,H_2O(l)$ = 6 *D*-glucose(aq),

β-cyclodextrin(aq) + $7\,H_2O(l)$ = 7 *D*-glucose(aq),

γ-cyclodextrin(aq) + $8\,H_2O(l)$ = 8 *D*-glucose(aq).

Equilibrium constants were determined for the following generalized cyclization reactions ($T = 329.6$ K, 0.005 mol kg^{-1} K_2HPO_4 buffer adjusted to pH = 5.55 with H_3PO_4) catalyzed by cyclomaltodextrin glucanotransferase:

G_u(aq) = α-cyclodextrin(aq) + $G_{(u-6)}$(aq),

G_v(aq) = β-cyclodextrin(aq) + $G_{(v-7)}$(aq),

G_w(aq) = γ-cyclodextrin(aq) + $G_{(w-8)}$(aq).

Here, G_1 is D-glucose and the G_n's (n is a positive integer) are linear maltodextrins; u, v, and w are, respectively, integers ≥ 7, ≥ 8, and ≥ 9. Values of the equilibrium constants, standard molar Gibbs energy change $\Delta_r G_m^\circ$, standard molar enthalpy change $\Delta_r H_m^\circ$, standard molar entropy change $\Delta_r S_m^\circ$, and standard molar heat-capacity change $\Delta_r C_{p,m}^\circ$ are tabulated for the above reactions at $T = 298.15$ K. The values of $\Delta_r G_m^\circ$ and $\Delta_r S_m^\circ$ for the first three above-mentioned reactions rely upon an estimated value of $\Delta_r S_m^\circ$ for the hydrolysis reaction of maltose to D-glucose. The thermodynamics of the disproportionation reaction

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$G_m(aq) + G_n(aq) = G_{m-1}(aq) + G_{n+1}(aq)$

is also discussed. Values of the quantities $\Delta_r H_m^\circ/N$, $\Delta_r G_m^\circ/N$, $\Delta_r S_m^\circ/N$, and $\Delta_r C_{p,m}^\circ/N$ for the three above-mentioned hydrolysis reactions where N is the number of (1 → 4)-α-D-glucosidic bonds broken in each of these reactions, have been calculated and compared with thermodynamic quantities for the similar hydrolysis reaction of a linear oligosaccharide.

Keywords: α-, β-, and γ-Cyclodextrin; Cyclomaltodextrin glucanotransferase; Enthalpies of reaction; Equilibrium constants; Thermodynamics

1. Introduction

Central to any discussion of the thermodynamics of the cyclodextrins is a knowledge of the equilibrium constants K and the standard molar enthalpy changes $\Delta_r H_m^\circ$ for the following reactions:

$$G_7(aq) = \alpha\text{-cyclodextrin(aq)} + G_1(aq), \quad (1)$$

$$G_8(aq) = \beta\text{-cyclodextrin(aq)} + G_1(aq), \quad (2)$$

$$G_9(aq) = \gamma\text{-cyclodextrin(aq)} + G_1(aq), \quad (3)$$

$$G_m(aq) + G_n(aq) = G_{m-1}(aq) + G_{n+1}(aq), \quad (4)$$

$$\alpha\text{-cyclodextrin(aq)} + 6\,H_2O(l) = 6\,\text{D-glucose(aq)}, \quad (5)$$

$$\beta\text{-cyclodextrin(aq)} + 7\,H_2O(l) = 7\,\text{D-glucose(aq)}, \quad (6)$$

$$\gamma\text{-cyclodextrin(aq)} + 8\,H_2O(l) = 8\,\text{D-glucose(aq)}. \quad (7)$$

The cyclization reactions (1)–(3) and the disproportionation reaction (4) are catalyzed by the enzyme cyclomaltodextrin glucanotransferase (EC 2.4.1.19). The latter reaction serves to produce a distribution of the linear maltodextrins D-glucose (G_1), maltose (G_2), maltotriose (G_3), maltotetraose (G_4), maltopentaose (G_5),..., G_m, and G_n, where m and n are positive integers. The hydrolysis reactions (5)–(7) can be carried out rapidly using either cyclomaltodextrin glucanotransferase or alpha amylase (EC 3.2.1.1) to break open the cyclodextrin rings; glucan (1 → 4)-α-glucosidase (EC 3.2.1.3) is then used to cleave the (1 → 4)-α-D-glucosidic bonds.

The principal aim of this study is the determination of accurate values of thermodynamic quantities for the above reactions. There is also a substantial amount of industrial interest in cyclodextrins which are of increasing importance to the technology of drug delivery systems, separations, and foods [1,2]. Since cyclodextrins are manufactured from starch using cyclomaltodextrin glucanotransferase, the information obtained herein can have implications for the various processes used to produce these and related dextrins.

The previous literature in this area is limited to two earlier studies. Pazur [3] used paper chromatography to determine concentrations of D-glucose, maltose, maltotriose, maltotetraose, maltopentaose, and of α-, β-, and γ-cyclodextrin. He also calculated equilibrium constants for the cyclization reactions (1)–(3) and obtained results for the disproportionation reaction (4). Pazur's results, however, were obtained ≈ 46 years ago and, given the significant advances in methods of analysis since that time, it seemed worthwhile to perform measurements of equilibrium constants for reactions (1)–(3). Takahashi and Ono [4] used calorimetry to determine standard molar enthalpy changes for reactions (5)–(7). However, since their results are the only values in the literature and since they used a buffer (acetate) which interacts [5] with the cyclodextrins, it was also decided to repeat the measurements of these enthalpies of hydrolysis using a buffer (phosphate) which is known [5] to have negligible interaction with the cyclodextrins.

2. Experimental

Materials.—The principal substances used in this study, their respective Chemical Abstracts Services (CAS) registry numbers, and suppliers [1] (P = Pfanstiehl (Waukegan, IL), and S = Sigma (St. Louis, MO)) are: α-cyclodextrin, 10016-20-3, S; β-cyclodextrin, 7585-39-9, S; γ-cyclodextrin, 17465-86-0, S; maltose monohydrate, 6363-53-7, P; maltotriose,

[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.

1109-28-0, S; maltotetraose, 34612-38-9, S; maltopentaose, 34620-76-3, S; maltohexaose, 34620-77-4, S; maltoheptaose, 34620-78-5, S; K_2HPO_4, 7758-11-4, S. The D-glucose (CAS registry number 50-99-7) and the KH_2PO_4 (CAS registry number 7778-77-0) were Standard Reference Materials from the National Institute of Standards and Technology. The mole fraction purities of the α-, β-, and γ-cyclodextrin were found to be 0.985, 0.980, and 0.983, respectively, using a chromatographic procedure that was slightly modified from that described below. It was also found that: the α-cyclodextrin contained some β-cyclodextrin (mole fraction $x = 0.0015$); the β-cyclodextrin contained some α-cyclodextrin ($x = 0.00035$) and some γ-cyclodextrin ($x = 0.00013$); and the γ-cyclodextrin contained some β-cyclodextrin ($x = 0.0026$). Based upon their retention times, it is judged likely that the other impurities in these samples are D-glucose and other simple sugars. The mole fraction purities of the other oligosaccharides were judged to be > 0.995 using a chromatographic procedure similar to that described below. The mass fraction moisture contents of the carbohydrates as determined by Karl–Fischer analysis were: α-cyclodextrin, (0.110 ± 0.014); β-cyclodextrin, (0.124 ± 0.004); γ-cyclodextrin, (0.089 ± 0.007); D-glucose, (0.0016 ± 0.0002); maltose, (0.060 ± 0.002); maltotriose, (0.039 ± 0.005); maltotetraose, (0.056 ± 0.003); maltopentaose, (0.039 ± 0.003); maltohexaose, (0.054 ± 0.003); and maltoheptaose, (0.048 ± 0.004). The uncertainties given here and elsewhere in this paper are based on two estimated standard deviations of the mean unless stated otherwise. The relative molar masses of the carbohydrates are: D-glucose, 0.18016 kg mol^{-1}; maltose, 0.34231 kg mol^{-1}; maltotriose, 0.50444 kg mol^{-1}; maltotetraose, 0.66658 kg mol^{-1}; maltopentaose, 0.82873 kg mol^{-1}; maltohexaose, 0.99087 kg mol^{-1}; maltoheptaose, 1.1530 kg mol^{-1}; α-cyclodextrin, 0.97285 kg mol^{-1}; β-cyclodextrin, 1.1350 kg mol^{-1}; and γ-cyclodextrin, 1.2971 kg mol^{-1}.

The enzymes used in this study were alpha-amylase from *Bacillus species*, glucan $(1 \rightarrow 4)$-α-glucosidase from *Aspergillus niger*, and cyclomaltodextrin glucanotransferase from *Thermoanaerobacter*. The alpha amylase and the glucan $(1 \rightarrow 4)$-α-glucosidase, in the form of lyophilized powders, were from Sigma. The cyclomaltodextrin glucanotransferase was kindly provided by Mr. Don Duhart of Novo Nordisk BioChem (Franklinton, NC). This enzyme, which was intended for industrial use, was in a solution containing low molecular weight stabilizers. The mass fraction of protein (determined by lyophilization following dialysis against water) in the original solution was ≈ 0.028. Prior to its use, the cyclomaltodextrin glucanotransferase was dialyzed (molecular weight cutoff = 12 to 14 kDa) against the phosphate buffer used for that particular calorimetric or equilibrium experiment.

Chromatography.—The determination of the molalities of the carbohydrates in solution was performed with a Dionex HPLC equipped with a pulsed amperometric detector and a CarboPac PA1 column. Solutions I and II were used for the mobile phase: I, NaOH (0.1 mol dm^{-3}); and II, {NaOH (0.1 mol dm^{-3}) + NaOAc (0.6 mol dm^{-3})}. The following linear gradient was used: at time $t = 0$, solution I (volume fraction $\phi = 0.90$) and solution II ($\phi = 0.10$); at $t = 38$ min, the mobile phase consisted of solution I ($\phi = 0.30$) and solution II ($\phi = 0.70$). The flow rate was 0.6 cm^3 min^{-1}. The column was allowed to equilibrate for ≥ 10 min under initial gradient conditions prior to the next injection. Typical retention times were: D-glucose, 4.3 min; maltose, 8.5 min; maltotriose, 13.2 min; α-cyclodextrin, 15.6 min; maltotetraose, 16.3 min; maltopentaose, 18.6 min; maltohexaose, 20.6 min; maltoheptaose, 22.4 min; γ-cyclodextrin, 24.2 min; and β-cyclodextrin, 26.8 min.

All of the chromatographic peaks were baseline separated with the exceptions of the α-cyclodextrin and maltotetraose peaks and the γ-cyclodextrin peak which fell between two small unidentified peaks. The overlap of the α-cyclodextrin and maltotetraose peaks was slight and the peaks were of comparable size. Consequently, we judged that there was no need to resolve these two peaks numerically. However, the overlap of the γ-cyclodextrin peak with its neighboring peaks was significant. Therefore, these peaks were resolved numerically (PeakFit, Jandel Scientific Software, San Rafael, CA). Interestingly, the results obtained for the molalities m of γ-cyclodextrin with the peak resolution software differed by only $0.10 \cdot m(\gamma\text{-cyclodextrin})$ from the molalities obtained with the standard chromatographic software (Dionex, Sunnyvale, CA) in which a straight vertical line was dropped from the valley between the two overlapping peaks. Nevertheless, the area of the γ-cyclodextrin peak was the most difficult of all the areas to obtain. This was due not only to the need to resolve it from overlapping peaks but also because the calculated area was sensitive to the somewhat arbitrary choice of the baseline. For these reasons, we judge that a reasonable estimate of uncertainty in the molality of

the γ-cyclodextrin is $\approx 0.15 \cdot m(\gamma\text{-cyclodextrin})$. Systematic errors in the molalities of the other substances are judged to be $< 0.02 \cdot m$.

Equilibrium measurements.—Equilibrium constants for reactions (1)–(3) were determined by chromatographic measurement of the molalities of the pertinent substances in two separate solutions (A and B) in which equilibrium was approached from two different directions of reaction. Solution A contained D-glucose and β-cyclodextrin; solution B contained maltose, maltotriose, and maltoheptaose. Dilute phosphate buffer (0.005 mol kg^{-1} K_2HPO_4 adjusted to pH = 5.55 with H_3PO_4) was used for both solutions. Cyclomaltodextrin glucanotransferase was then added to both solutions. These reaction mixtures, contained in Erlenmeyer flasks, were shaken at ≈ 50 rpm in a water bath thermostatted at the temperature $T = 329.6$ K and allowed to equilibrate. The compositions of the two solutions were measured periodically using the HPLC procedure described above. It was deemed necessary to add some additional cyclomaltodextrin glucanotransferase during the 13 day equilibration period to achieve equilibrium as evidenced by agreement of the equilibrium constants calculated from the results obtained for both solutions A and B.

The γ-cyclodextrin peak in the chromatograms of these reaction mixtures was initially identified by its retention time relative to maltoheptaose and β-cyclodextrin. Its identification was confirmed by addition of an appropriate amount of γ-cyclodextrin to the reaction mixtures.

Calorimetry.—Three heat-conduction microcalorimeters were used for the enthalpy of reaction measurements. They were calibrated electrically with a high stability d.c. power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results are given in references [6] and [7]. The sample vessels, fabricated from high-density polyethylene, contained two compartments that held ≈ 0.55 cm^3 and ≈ 0.45 cm^3 of solution. The substrate solution (placed in the 0.55 cm^3 compartment) consisted of either α-, β-, or γ-cyclodextrin dissolved in KH_2PO_4 buffer ($m = 0.10$ mol kg^{-1}, pH = 4.5). In the calorimetric experiments, glucan (1 → 4)-α-glucosidase and alpha amylase were used for the hydrolysis of β- and γ-cyclodextrin; glucan (1 → 4)-α-glucosidase and cyclomaltodextrin glucanotransferase were used for the hydrolysis of α-cyclodextrin. The enzyme solution, which consisted of glucan (1 → 4)-α-glucosidase and either alpha amylase or cyclomaltodextrin glucanotransferase dissolved in the same KH_2PO_4 buffer, was placed in the 0.45 cm^3 compartment.

The microcalorimeter vessels and their contents were allowed to equilibrate in the calorimeters for 1 hour before the enzyme and substrate solutions were mixed. Following reaction in the calorimeter for 30 to 50 min, the reaction vessels were removed from the calorimeters and their contents were promptly analyzed with the Dionex HPLC. Thus, it was found that the mole fractions of unreacted α-, β-, and γ-cyclodextrin were, respectively, 0.027, 0.0014, and 0.0042. Appropriate corrections were applied for incomplete reaction. The 'blank' enthalpy changes for the mixing of the substrate solutions with the buffer ranged from −0.1 mJ to 2.1 mJ; for the mixing of the enzyme solutions with the buffer they ranged from −1.3 mJ to 0.5 mJ. These 'blank' enthalpies of mixing were also applied as corrections to the measured enthalpy changes which were approximately −235 mJ, −200 mJ, and −175 mJ for the respective reactions involving α-, β-, and γ-cyclodextrin. Appropriate corrections were also applied for the impurities in the cyclodextrins. In applying these latter corrections, it was assumed that, with the exception of the cyclodextrin impurities, these impurities were 'inert' reactants and made no contribution to the measured enthalpy changes.

3. Results and discussion

Thermodynamic formalism.—The equilibrium constants that correspond, respectively, to reactions (1)–(7) are:

$$K(1) = m(\alpha\text{-cyclodextrin}) \cdot m(G_1)/m(G_7), \quad (8)$$

$$K(2) = m(\beta\text{-cyclodextrin}) \cdot m(G_1)/m(G_8), \quad (9)$$

$$K(3) = m(\gamma\text{-cyclodextrin}) \cdot m(G_1)/m(G_9), \quad (10)$$

$$K(4) = \{m(G_{m-1}) \cdot m(G_{n+1})\}/\{m(G_m) \cdot m(G_n)\}, \quad (11)$$

$$K(5) = \{m(\text{D-glucose})\}^6 / \{m(\alpha\text{-cyclodextrin}) \cdot (m^\circ)^5\}, \quad (12)$$

$$K(6) = \{m(\text{D-glucose})\}^7 / \{m(\beta\text{-cyclodextrin}) \cdot (m^\circ)^6\}, \quad (13)$$

$$K(7) = \{m(\text{D-glucose})\}^8$$

$$/\left\{m(\gamma\text{-cyclodextrin}) \cdot (m^\circ)^7\right\}. \quad (14)$$

We have taken the standard state of the solutes as the hypothetical ideal solution of unit molality ($m^\circ = 1$ mol kg^{-1}) and the activity of water as equal to unity. The m°'s have been placed in the denominators of Eqs. (12)–(14) so that the equilibrium constants will be dimensionless.

In considering the thermodynamics of these reactions it was found useful to follow a formalism like that used by Pazur [3] and which is now described. Let P be the probability of forming a given (1 → 4)-α-D-glucosidic bond and $(1 - P)$ be the probability of that bond not being formed. In the following discussion, we shall assume that P is independent of chain length. The evidence for the correctness of this assumption is discussed later in this paper. Thus, the probability P_n of obtaining the molecule G_n which contains $(n - 1)$ bonds is

$$P_n = P^{(n-1)} \cdot (1 - P). \quad (15)$$

Consequently, in dilute solutions, the molality of the substance G_n in solution will be proportional to P_n and

$$m(G_n) = A_1 \cdot P^{(n-1)} \cdot (1 - P), \quad (16)$$

where A_1 is a constant. Accordingly,

$$\ln\{m(G_n)/m^\circ\} = \ln(A_1) + \ln(1 - P) - \ln(P) + n \cdot \ln(P), \quad (17)$$

$$\ln\{m(G_n)/m^\circ\} = A_2 + n \cdot \ln(P), \quad (18)$$

where the constant $A_2 = \ln(A_1) + \ln(1 - P) - \ln(P)$. Thus, it is predicted that a plot of $\ln\{m(G_n)/m^\circ\}$ versus n will yield a straight line with a slope equal to $\ln(P)$. Use of Eq. (16) leads to

$$m(G_n)/m(G_{n+j}) = P^{-j}, \quad (19)$$

where j is an integer. Comparison of Eq. (19) with Pazur's [3] Eq. (11) shows that Pazur's 'i' parameter is equal to $(1 - P)$.

Substitution of Eq. (19) in Eq. (11) leads to the result that the equilibrium constant for the disproportionation reaction (4) is equal to 1. This result is independent of the value of P and it holds for all temperatures. Consequently, the standard molar Gibbs energy change $\Delta_r G_m^\circ$, the standard molar enthalpy change $\Delta_r H_m^\circ$, the standard molar entropy change $\Delta_r S_m^\circ$, and the standard molar heat-capacity change $\Delta_r C_{p,m}^\circ$ for the disproportionation reaction (4) are all equal to zero. These respective reaction quantities will also be equal to zero if the individual standard molar Gibbs energies of formation, enthalpies of formation, partial molar entropies, and partial molar heat capacities of the substances G_n are linear functions of n.

We now consider the disproportionation reactions

$$G_u(\text{aq}) + G_1(\text{aq}) = G_7(\text{aq}) + G_{(u-6)}(\text{aq}), \quad (20)$$

$$G_v(\text{aq}) + G_1(\text{aq}) = G_8(\text{aq}) + G_{(v-7)}(\text{aq}), \quad (21)$$

$$G_w(\text{aq}) + G_1(\text{aq}) = G_9(\text{aq}) + G_{(w-8)}(\text{aq}), \quad (22)$$

where u, v, and w are, respectively, integers ≥ 7, ≥ 8, and ≥ 9. Addition of these respective disproportionation reactions to reactions (1)–(3), respectively, gives the generalized cyclization reactions

$$G_u(\text{aq}) = \alpha\text{-cyclodextrin(aq)} + G_{(u-6)}(\text{aq}), \quad (23)$$

$$G_v(\text{aq}) = \beta\text{-cyclodextrin(aq)} + G_{(v-7)}(\text{aq}), \quad (24)$$

$$G_w(\text{aq}) = \gamma\text{-cyclodextrin(aq)} + G_{(w-8)}(\text{aq}). \quad (25)$$

The equilibrium constants corresponding to these respective reactions are

$$K(23) = m(\alpha\text{-cyclodextrin}) \cdot m(G_{u-6})/m(G_u), \quad (26)$$

$$K(24) = m(\beta\text{-cyclodextrin}) \cdot m(G_{v-7})/m(G_v), \quad (27)$$

$$K(25) = m(\gamma\text{-cyclodextrin}) \cdot m(G_{w-8})/m(G_w). \quad (28)$$

Since $\Delta_r G_m^\circ = 0$ for the disproportionation reactions,

$$\Delta_r G_m^\circ(1) = \Delta_r G_m^\circ(23), \quad (29)$$

$$\Delta_r G_m^\circ(2) = \Delta_r G_m^\circ(24), \quad (30)$$

$$\Delta_r G_m^\circ(3) = \Delta_r G_m^\circ(25). \quad (31)$$

Thus, $K(1) = K(23)$, $K(2) = K(24)$, and $K(3) = K(25)$. Use of Eq. (19) with, respectively, Eqs. (26)–(28) leads to

$$K(23) = m(\alpha\text{-cyclodextrin}) \cdot P^{-6}, \quad (32)$$

$$K(24) = m(\beta\text{-cyclodextrin}) \cdot P^{-7}, \quad (33)$$

$$K(25) = m(\gamma\text{-cyclodextrin}) \cdot P^{-8}. \quad (34)$$

Since the quantities $\Delta_r H_m^\circ$, $\Delta_r S_m^\circ$, $\Delta_r C_{p,m}^\circ$ are equal to zero for the disproportionation reactions, it follows that equations similar to Eqs. (29)–(31) also hold for these thermodynamic quantities. In summary, the above treatment allows one to deal with the thermodynamics of the generalized cyclization reactions (23)–(25) rather than deal with a series of individual reactions similar to reactions (1)–(3). Additionally, the equilibrium constants for these reactions are con-

veniently calculated with Eqs. (32)–(34).

Results of equilibrium experiments.—The molalities of the substrates in solution following a 13 day equilibration at $T = 329.6$ K in the pH = 5.55 ($K_2HPO_4 + H_3PO_4$) buffer containing cyclomaltodextrin glucanotransferase are given in Table 1. The results are well represented by straight lines in plots of $\ln\{m(G_n)/m^\circ\}$ versus n (see Fig. 1). With Eq. (18) we calculate $P = (0.564 \pm 0.014)$ and $P = (0.515 \pm 0.012)$ from the results for solutions A and B, respectively. Then with Eqs. (32)–(34) we obtain: $K(23) = (0.0221 \pm 0.0038)$, $K(24) = (0.0397 \pm 0.0081)$, and $K(25) = (0.0105 \pm 0.0037)$ from the results for solution A; $K(23) = (0.0242 \pm 0.0051)$, $K(24) = (0.0383 \pm 0.0078)$, and $K(25) = (0.0101 \pm 0.0035)$ from the results for solution B. Thus, we adopt the following weighted average equilibrium constants from the two different directions of reaction: $K(23) = (0.0229 \pm 0.0030)$, $K(24) = (0.0390 \pm 0.0056)$, and $K(25) = (0.0103 \pm 0.0025)$ at $T = 329.6$ K. The cyclization reactions are judged to be at equilibrium as evidenced by agreement of the results obtained from different directions of reaction. Therefore, no estimate is made for systematic errors due to a failure to reach equilibrium.

An alternative method is to calculate values of $K(1)$, $K(2)$, and $K(3)$ directly from the molalities of the substances in solution. This requires values for the molalities of maltooctaose (G_8) and maltononaose (G_9), which were not measured. However, these molalities can be calculated with Eq. (18) and the values of the slopes and intercepts obtained above. Thus,

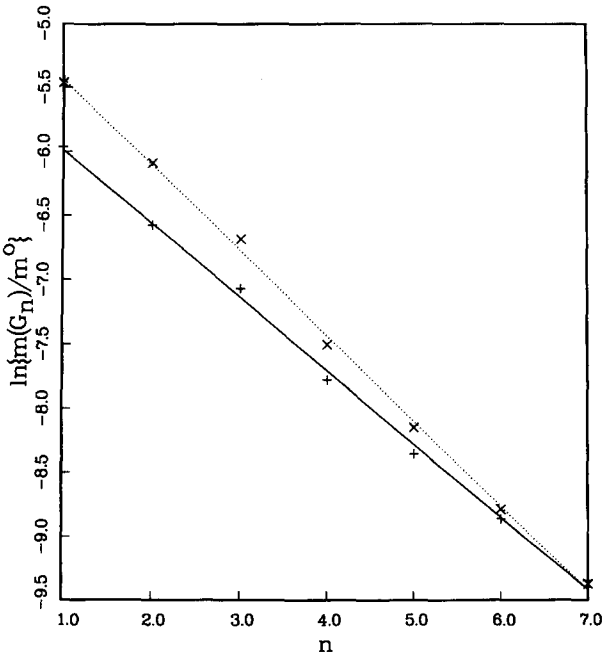

Fig. 1. Plot of $\ln\{m(G_n)/m^\circ\}$ versus n. For solution A (+ connected with the solid line) the results are: slope = $-(0.572 \pm 0.024)$, intercept = $-(5.42 \pm 0.11)$, and residual standard deviation = 0.063. For solution B (X connected with the dotted line), the results are: slope = $-(0.664 \pm 0.023)$, intercept = $-(4.78 \pm 0.10)$, and residual standard deviation = 0.060.

$m(G_8) = (0.0459 \pm 0.010) \cdot 10^{-3}$ mol kg^{-1} and $m(G_9) = (0.0259 \pm 0.0063) \cdot 10^{-3}$ mol kg^{-1} for solution A; $m(G_8) = (0.0417 \pm 0.0088) \cdot 10^{-3}$ mol kg^{-1} and $m(G_9) = (0.0215 \pm 0.0050) \cdot 10^{-3}$ mol

Table 1
Molalities m of substrates in solution following a 13 day equilibration at $T = 329.6$ K in phosphate buffer (0.005 mol kg^{-1} K_2HPO_4 adjusted to pH = 5.55 with H_3PO_4) containing cyclomaltodextrin glucanotransferase (mass fraction ≈ 0.0014)

Solution	$10^3 \cdot m(G_1)$ /(mol kg^{-1}) [a,b]	$10^3 \cdot m(G_2)$ /(mol kg^{-1})	$10^3 \cdot m(G_3)$ /(mol kg^{-1})	$10^3 \cdot m(G_4)$ /(mol kg^{-1})	$10^3 \cdot m(G_5)$ /(mol kg^{-1})
A [c]	2.57 ± 0.08	1.40 ± 0.05	0.856 ± 0.023	0.422 ± 0.013	0.237 ± 0.009
B [c]	4.24 ± 0.15	2.28 ± 0.08	1.260 ± 0.056	0.556 ± 0.026	0.292 ± 0.014
Solution	$10^3 \cdot m(G_6)$ /(mol kg^{-1})	$10^3 \cdot m(G_7)$ /(mol kg^{-1})	$10^3 \cdot m(\alpha\text{-CD})$ /(mol kg^{-1})	$10^3 \cdot m(\beta\text{-CD})$ /(mol kg^{-1})	$10^3 \cdot m(\gamma\text{-CD})$ /(mol kg^{-1})
A	0.143 ± 0.006	0.087 ± 0.005	0.710 ± 0.017	0.720 ± 0.022	0.107 ± 0.016
B	0.154 ± 0.008	0.085 ± 0.005	0.452 ± 0.032	0.368 ± 0.015	0.050 ± 0.008

[a] Abbreviations are: G_1, D-glucose; G_2, maltose; G_3, maltotriose; G_4, maltotetraose; G_5, maltopentaose; G_6, maltohexaose; G_7, maltoheptaose; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; and γ-CD, γ-cyclodextrin.

[b] The uncertainties in the molalities are based on a combination (quadrature) of random errors and estimates of possible systematic errors in the chromatography. The random errors are based on two estimated standard deviations of the mean and are calculated from the standard deviations of the chromatographic areas obtained in the determination of the response factors and of the molalities of these substrates in solution. The systematic errors in the molalities of the substrates are judged to be $< 0.02 \cdot m$ except for $m(\gamma\text{-CD})$ where allowance is made for a possible systematic error of $0.15 \cdot m$ (see Section 2, Experimental).

[c] Initially, solution A contained only D-glucose and β-cyclodextrin while solution B contained only maltose, maltotriose, and maltoheptaose.

kg^{-1} for solution B. Using these calculated molalities and the other requisite molalities from Table 1, we obtain $K(1) = (0.0210 \pm 0.0030)$, $K(2) = (0.040 \pm 0.011)$, and $K(3) = (0.0106 \pm 0.0045)$ from the solution A results and $K(1) = (0.0225 \pm 0.0037)$, $K(2) = (0.037 \pm 0.011)$, and $K(3) = (0.0099 \pm 0.0042)$ from the solution B results. The weighted average equilibrium constants from the two different directions of reaction are: $K(1) = (0.0218 \pm 0.0020)$, $K(2) = (0.0385 \pm 0.0078)$, and $K(3) = (0.0102 \pm 0.0031)$ at $T = 329.6$ K. It is seen that this alternative method of calculation leads to values for the equilibrium constants that are in agreement with the above method which used Eqs. (32)–(34). Nevertheless, the results obtained with Eqs. (32)–(34) will be used in all subsequent calculations.

We use the values of $\Delta_r H_m^\circ$ and $\Delta_r C_{p,m}^\circ$ for reactions (23)–(25) which are obtained later in this paper (see Table 3) to adjust these equilibrium constants to $T = 298.15$ K. Thus, we obtain $K(23) = (0.0103 \pm 0.0013)$, $K(24) = (0.0216 \pm 0.0031)$, and $K(25) = (0.0059 \pm 0.0015)$ at the reference temperature of 298.15 K. The corresponding standard molar Gibbs energies of reaction are $\Delta_r G_m^\circ(23) = (11.3 \pm$

Table 2
Results of calorimetric measurements for reactions (5)–(7) in aqueous KH_2PO_4 buffer at $T = 298.15$ K and ionic strength 0.10 mol kg^{-1}

Reaction (5): [a] α-cyclodextrin(aq) + 6 H_2O(l) = 6 D-glucose(aq)				
Experiment	pH [b]	$m(KH_2PO_4)$/(mol kg^{-1}) [c]	m(α-cyclodextrin)/(mol kg^{-1})	$\Delta_r H_m$(cal)/(kJ mol^{-1}) [d,e]
1	4.58	0.101	0.00508	−50.88
2	4.58	0.101	0.00494	−51.71
3	4.58	0.101	0.00524	−50.70
4	4.58	0.101	0.00528	−51.51
5	4.58	0.101	0.00543	−50.88
6	4.58	0.101	0.00533	−50.76
7	4.58	0.101	0.00545	−49.83
8	4.58	0.101	0.00547	−50.53
		$\langle \Delta_r H_m(\text{cal}) \rangle =$	$-(50.85 \pm 0.40)$ kJ mol^{-1}	
Reaction (6): [f] β-cyclodextrin(aq) + 7 H_2O(l) = 7 D-glucose(aq)				
Experiment	pH	$m(KH_2PO_4)$/(mol kg^{-1})	m(β-cyclodextrin)/(mol kg^{-1})	$\Delta_r H_m$(cal)/(kJ mol^{-1})
1	5.14	0.101	0.00448	−48.37
2	5.14	0.101	0.00435	−49.05
3	5.14	0.101	0.00424	−49.02
4	5.14	0.101	0.00445	−48.63
5	5.14	0.101	0.00459	−48.76
6	5.14	0.101	0.00433	−48.88
		$\langle \Delta_r H_m(\text{cal}) \rangle =$	$-(48.79 \pm 0.21)$ kJ mol^{-1}	
Reaction (7): [f] γ-cyclodextrin(aq) + 8 H_2O(l) = 8 D-glucose(aq)				
Experiment	pH	$m(KH_2PO_4)$/(mol kg^{-1})	m(γ-cyclodextrin)/(mol kg^{-1})	$\Delta_r H_m$(cal)/(kJ mol^{-1})
1	5.15	0.101	0.00374	−51.32
2	5.15	0.101	0.00363	−51.17
3	5.15	0.101	0.00359	−53.20
4	5.15	0.101	0.00336	−52.53
5	5.15	0.101	0.00342	−51.92
6	5.15	0.101	0.00358	−53.61
		$\langle \Delta_r H_m(\text{cal}) \rangle =$	$-(52.29 \pm 0.81)$ kJ mol^{-1}	

[a] The respective mass fractions of the glucan (1 → 4)-α-glucosidase and cyclomaltodextrin glucanotransferase in the solutions used for reaction (5) were 0.0012 and 0.013.
[b] The pHs are those of the final reaction mixtures. The alpha amylase brought about changes of ≈ 0.65 in the pHs of the solutions used for reactions (6) and (7).
[c] The molalities m are those obtained after mixing of the enzyme and substrate solutions and prior to any reaction.
[d] $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction.
[e] The uncertainties are equal to two estimated standard deviations of the mean.
[f] The respective mass fractions of the glucan (1 → 4)-α-glucosidase and alpha amylase in the solutions used for reactions (6) and (7) were 0.0012 and 0.0063.

0.3) kJ mol^{-1}, $\Delta_r G_m°(24) = (9.5 \pm 0.4)$ kJ mol^{-1}, and $\Delta_r G_m°(25) = (12.7 \pm 0.6)$ kJ mol^{-1}.

Calorimetric results.—Results for the calorimetrically determined molar enthalpies $\Delta_r H_m$(cal) for reactions (5)–(7) are given in Table 2. Since the carbohydrates involved in these reactions do not ionize unless placed in extremely alkaline solutions, there is no need to apply any buffer protonation or ionization corrections to the results [8,9]. Also, any corrections for enthalpies of dilution are judged to be small (< 0.2 kJ mol^{-1}) since these reactants all have a charge number of zero. Thus, $\Delta_r H_m°$ at ionic strength $I = 0$ is taken to be equal to $\Delta_r H_m$(cal) for each respective reaction.

Errors in the calorimetry are judged to contribute $< 0.005 \cdot \Delta_r H_m(\text{cal}) \approx 0.25$ kJ mol^{-1}, with the major portion of this error arising from the uncertainty in the measurements of the 'blank' enthalpies. We judge the corrections for the impurities in the samples to introduce systematic errors < 0.33 times the value of the correction; this leads to possible systematic errors in the range 0.25 kJ mol^{-1} to 0.33 kJ mol^{-1} in the values of $\Delta_r H_m$(cal). The corrections for incomplete conversion of the cyclodextrins are judged to introduce systematic errors < 0.25 times the value of this correction. In this case, the largest possible systematic error (0.34 kJ mol^{-1}) pertains to the hydrolysis of α-cyclodextrin. Possible systematic errors due to the moisture corrections are judged to be $< s \cdot \Delta_r H_m$(cal) where s is the estimated standard deviation of the mean of the mass fraction moisture content of the cyclodextrin as determined by Karl–Fischer analysis. Finally, as mentioned above, neglect of any corrections for enthalpies of dilution are judged to be < 0.2 kJ mol^{-1}. These estimates of systematic error are combined in quadrature together with the statistical uncertainties in the measured values of $\Delta_r H_m$(cal), expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties of 0.59 kJ mol^{-1}, 0.47 kJ mol^{-1}, and 0.60 kJ mol^{-1} for the values of $\Delta_r H_m$(cal) for reactions (5)–(7), respectively. These combined standard uncertainties are then multiplied by two to arrive at a final set of estimates of uncertainties in the results. Thus, we have $\Delta_r H_m°(5) = -(50.9 \pm 1.2)$ kJ mol^{-1}, $\Delta_r H_m°(6) = -(48.8 \pm 1.0)$ kJ mol^{-1}, and $\Delta_r H_m°(7) = -(52.3 \pm 1.2)$ kJ mol^{-1} at $T = 298.15$ K.

Previous literature.—Pazur [3] reported (see his Tables 7 and 8) concentrations of D-glucose, maltose, maltotriose, maltotetraose, and α-, β-, and γ-cyclodextrin following equilibration with cyclomaltodextrin glucanotransferase obtained from *Bacillus macerans*. The initial reaction solution contained maltoheptaose dissolved in a buffered solution (0.001 mol dm^{-3} NaCN adjusted to pH = 6.5 with sodium acetate). The temperature of reaction was 311.15 K. We have recalculated Pazur's results [3] by fitting Eq. (18) to his reported concentrations of D-glucose, maltose, maltotriose, and maltotetraose. A value of $P = (0.711 \pm 0.023)$ was obtained from this fit and then used with the reported concentrations of the cyclodextrins in reactions (32)–(34) to calculate the following values of the equilibrium constants at $T = 311.15$ K: $K(23) = 0.0134$, $K(24) = 0.0334$, and $K(25) = 0.0194$. The alternative method of calculation where values of $m(G_8)$ and $m(G_9)$ are calculated was also used. This method yielded the same values for these equilibrium constants. Comparison of the values of the equilibrium constants recalculated from Pazur's results with the values of these equilibrium constants determined in the present study require adjustment to a common temperature. We do this with values of $\Delta_r H_m°$ and $\Delta_r C_{p,m}°$ for reactions (23)–(25) which are obtained later in this paper and which are given in Table 3. Thus, the equilibrium constants at $T = 298.15$ K calculated from Pazur's results are: $K(23) = 0.0092$, $K(24) = 0.0253$, and $K(25) = 0.0149$. Hence, it is seen that the values of the equilibrium constants recalculated from Pazur's results are in good agreement with the values of the equilibrium constants for reactions (23) and (24) from the current study. However, this is not the case for the equilibrium constant for reaction (25) which is the most difficult of the three equilibrium constants to measure. This difficulty exists primarily because the value of $K(25)$ relies upon a determination of the molality of γ-cyclodextrin which is the most difficult of the cyclodextrin molalities to measure. Also, any error in the value of P will cause a larger error in the value of $K(25)$ than for the other two equilibrium constants. However, we prefer the result for $K(25)$ obtained in the current study basically because it relies upon analytical methods that are much improved over those that were available to Pazur [3] in his early study.

Takahashi and Ono [4] performed calorimetric measurements and obtained $\Delta_r H_m° = -(53.5 \pm 4.7)$ kJ mol^{-1}, $-(48.7 \pm 2.0)$ kJ mol^{-1}, and $-(53.0 \pm 1.5)$ kJ mol^{-1} for reactions (5)–(7), respectively. The temperature of reaction was 298.15 K and acetate buffer (concentration $c = 0.02$ mol dm^{-3}, pH = 5.0) was used. The uncertainties given for these values of $\Delta_r H_m°$ are two estimated standard deviations of the mean and do not include any components for possible

Table 3
Thermodynamic quantities at $T = 298.15$ K for the several reactions studied herein [a]

No.	Reaction [b]	$\Delta_r H_m^\circ$/(kJ mol^{-1})	K	$\Delta_r G_m^\circ$/(kJ mol^{-1})	$\Delta_r S_m^\circ$/(J K^{-1} mol^{-1})	$\Delta_r C_{p,m}^\circ$/(J K^{-1} mol^{-1})
4	$G_m(aq) + G_n(aq) = G_{m-1}(aq) + G_{n+1}(aq)$	0 [c]	1.0 [c]	0 [c]	0 [c]	0 [c]
5	α-cyclodextrin(aq) + 6 H_2O(l) = 6 D-glucose(aq)	−(50.9 ± 1.2)	$2 \cdot 10^{19}$ [d]	−110 [d]	197 [d]	187 ± 15
6	β-cyclodextrin(aq) + 7 H_2O(l) = 7 D-glucose(aq)	−(48.8 ± 1.0)	$5 \cdot 10^{21}$ [d]	−124 [d]	253 [d]	105 ± 53
7	γ-cyclodextrin(aq) + 8 H_2O(l) = 8 D-glucose(aq)	−(52.3 ± 1.2)	$2 \cdot 10^{25}$ [d]	−144 [d]	308 [d]	88 ± 23
23	$G_u(aq)$ = α-cyclodextrin(aq) + $G_{(u-6)}(aq)$	24.1 ± 1.3	0.0103 ± 0.0013	11.3 ± 0.3	43 ± 5	−(221 ± 13)
24	$G_v(aq)$ = β-cyclodextrin(aq) + $G_{(v-7)}(aq)$	17.5 ± 1.1	0.0216 ± 0.0031	9.5 ± 0.4	27 ± 5	−(145 ± 53)
25	$G_w(aq)$ = γ-cyclodextrin(aq) + $G_{(w-8)}(aq)$	16.4 ± 1.3	0.0059 ± 0.0015	12.7 ± 0.6	12 ± 5	−(133 ± 21)

[a] The standard state is the hypothetical ideal solution of unit molality.
[b] The quantities m, n, u, v, and w are integers ($m \geq 2$, $n \geq 1$, $u \geq 7$, $v \geq 8$, $w \geq 9$).
[c] Based upon the assumption that P is independent of chain length (see Section 3, Results and discussion).
[d] These are approximate values based upon an estimated value of $\Delta_r S_m^\circ$ for reaction (37).

systematic errors, one of which is the possible interaction of the cyclodextrins with the acetate buffer [5]. In any case, it is seen that the values of $\Delta_r H_m^\circ$ for reactions (5)–(7) obtained from this study and from the study of Takahashi and Ono [4] are in agreement.

Basis of the assumption regarding the independence of the probability of forming a given (1 → 4)-α-D-glucosidic bond on chain length.—Evidence for the correctness of the assumption that P is independent of chain length comes from several directions. The first is the observed linearity of plots of $\ln\{G_n/\mathrm{m}^\circ\}$ versus n as seen both in this study (see Fig. 1) and in the study of Pazur [3]. These observations are in agreement with Eq. (18) which was based upon this assumption regarding P. Also, the molalities of the linear maltodextrins given in Table 1 can be used to calculate values of equilibrium constants for the disproportionation reaction (4). This calculation leads with the solution A and B results, respectively, to $\langle K(4)\rangle = (0.94 \pm 0.05)$ and $\langle K(4)\rangle = (0.98 \pm 0.06)$. The latter value is in agreement with the predictions of the assumption regarding P, while the former value is nearly in agreement. In performing this calculation, the value of $\langle K(4)\rangle$ would be exactly unity if it were calculated using all possible ways of writing $K(4)$. Therefore, only the equilibrium constants $K(4)$ that met the requirement $\{|m - n| > |m - n - 2|\}$ were used in the calculation of $\langle K(4)\rangle$.

Additional evidence for the correctness of the assumption regarding the independence of P on chain length comes from the study of Briggner and Wadsö [10] who measured values of the standard partial molar heat capacities $C_{p,2,m}^\circ$ of aqueous D-glucose, maltose, maltotriose, and maltotetraose (i.e. G_n for $n = 1$ to 4). These results are well represented by the equation

$$C_{p,2,m}^\circ(G_n)/\mathrm{J\ K^{-1}\ mol^{-1}} = a + b \cdot n, \qquad (35)$$

where $a = (71.0 \pm 3.8)$ and $b = (275.2 \pm 1.4)$. The residual standard deviation of the fit is 1.6 J $\mathrm{K^{-1}\ mol^{-1}}$. Similarly, Goldberg et al. [11] measured standard molar enthalpies of hydrolysis $\Delta_r H_m^\circ$ for the reactions ($n = 2$ to 7)

$$G_n(\mathrm{aq}) + (n-1)\mathrm{H_2O(l)} = nG_1(\mathrm{aq}). \qquad (36)$$

They [11] found that $\Delta_r H_m^\circ(36)/N$, where N is the number of (1 → 4)-α-D-glucosidic bonds broken in the hydrolysis reactions (36), was a constant equal to $-(4.53 \pm 0.04)$ kJ $\mathrm{mol^{-1}}$ at $T = 298.15$ K. Therefore, $\Delta_r H_m^\circ(36)$ is a linear function of n. As pointed out above, the thermodynamic quantities $\Delta_r G_m^\circ$, $\Delta_r H_m^\circ$, $\Delta_r S_m^\circ$, and $\Delta_r C_{p,m}^\circ$ for the disproportionation reaction (4) will be zero if the individual standard molar Gibbs energies of formation, enthalpies of formation, partial molar entropies, and partial molar heat capacities of the substances G_n are linear functions of n.

Summary thermodynamics.—We can now calculate thermodynamic quantities at $T = 298.15$ K for the cyclization reactions (23)–(25). The values of the $\Delta_r H_m^\circ$'s obtained in this study for the hydrolysis reactions (5)–(7) are combined with the appropriate $\Delta_r H_m^\circ$'s for the hydrolysis reactions (36) of the linear maltodextrins G_7, G_8, and G_9 [11] to give $\Delta_r H_m^\circ(23) = (24.1 \pm 1.3)$ kJ $\mathrm{mol^{-1}}$, $\Delta_r H_m^\circ(24) = (17.5 \pm 1.1)$ kJ $\mathrm{mol^{-1}}$, and $\Delta_r H_m^\circ(25) = (16.4 \pm 1.3)$ kJ $\mathrm{mol^{-1}}$. These $\Delta_r H_m^\circ$'s are combined with the $\Delta_r G_m^\circ$'s for these same reactions to yield $\Delta_r S_m^\circ(23) = (43 \pm 5)$ J $\mathrm{K^{-1}\ mol^{-1}}$, $\Delta_r S_m^\circ(24) = (27 \pm 5)$ J $\mathrm{K^{-1}\ mol^{-1}}$, and $\Delta_r S_m^\circ(25) = (12 \pm 6)$ J $\mathrm{K^{-1}\ mol^{-1}}$. Thus, there is a substantial entropic contribution to reactions (23)–(25), without which there would be only an infinitesimal amount of the cyclodextrins formed. We use the standard partial molar heat capacities of the aqueous cyclodextrins [10], of D-glucose [12], and of the linear maltodextrins G_7, G_8, and G_9 [these values are obtained with Eq. (35)] to calculate $\Delta_r C_{p,m}^\circ(23) = -(221 \pm 13)$ J $\mathrm{K^{-1}\ mol^{-1}}$, $\Delta_r C_{p,m}^\circ(24) = -(145 \pm 53)$ J $\mathrm{K^{-1}\ mol^{-1}}$, and $\Delta_r C_{p,m}^\circ(25) = -(133 \pm 21)$ J $\mathrm{K^{-1}\ mol^{-1}}$. The uncertainties in the values of the $\Delta_r C_{p,m}^\circ$'s are based primarily on the estimated uncertainties [10] in the values of the standard partial molar heat capacities of the aqueous cyclodextrins.

We can also obtain thermodynamic quantities for the hydrolysis reactions (5)–(7) at $T = 298.15$ K. The standard partial molar heat capacities of the aqueous cyclodextrins [10], of D-glucose [12], and of $\mathrm{H_2O(l)}$ [13] are used to calculate $\Delta_r C_{p,m}^\circ(5) = (187 \pm 15)$ J $\mathrm{K^{-1}\ mol^{-1}}$, $\Delta_r C_{p,m}^\circ(6) = (105 \pm 53)$ J $\mathrm{K^{-1}\ mol^{-1}}$, and $\Delta_r C_{p,m}^\circ(7) = (88 \pm 23)$ J $\mathrm{K^{-1}\ mol^{-1}}$. As above, the uncertainties in the values of the $\Delta_r C_{p,m}^\circ$'s are based primarily on the estimated uncertainties [10] in the values of the standard partial molar heat capacities of the aqueous cyclodextrins. However, an estimate is required to obtain values for $\Delta_r S_m^\circ$ and $\Delta_r G_m^\circ$ for these hydrolysis reactions. This estimate is based upon the finding of Tewari and Goldberg [14] that $\Delta_r S_m^\circ$ for the hydrolysis reactions of eight different disaccharides fall in a relatively narrow range of values (31 to 56 J $\mathrm{K^{-1}\ mol^{-1}}$) with $\langle \Delta_r S_m^\circ \rangle = 40$ J $\mathrm{K^{-1}\ mol^{-1}}$. On this basis, we estimate $\Delta_r S_m^\circ = 40$

J K^{-1} mol^{-1} for the reaction(s) ($n \geq 2$)

$$G_n(aq) + H_2O(l) = G_{n-1}(aq) + G_1(aq). \quad (37)$$

These estimated $\Delta_r S_m^\circ$'s are combined with the already calculated $\Delta_r S_m^\circ$'s for the cyclization reactions (23)–(25) to yield $\Delta_r S_m^\circ(5) \approx 197$ J K^{-1} mol^{-1}, $\Delta_r S_m^\circ(6) \approx 253$ J K^{-1} mol^{-1}, and $\Delta_r S_m^\circ(7) \approx 308$ J K^{-1} mol^{-1}. Combination of these $\Delta_r S_m^\circ$'s with the calorimetrically determined $\Delta_r H_m^\circ$'s leads to $\Delta_r G_m^\circ(5) \approx -110$ kJ mol^{-1}, $\Delta_r G_m^\circ(6) \approx -124$ kJ mol^{-1}, and $\Delta_r G_m^\circ(7) \approx -144$ kJ mol^{-1}. Thus, it is seen that, at equilibrium, the hydrolysis of the cyclodextrins to D-glucose via reactions (5)–(7) is, for all practical purposes, complete.

Thermodynamic quantities for the hydrolysis, cyclization, and disproportionation reactions are summarized in Table 3. These thermodynamic quantities can be used to calculate values (see Table 4) of $\Delta_r X_m^\circ/N$ ($X = G$, H, S, or C) where N is the number of $(1 \rightarrow 4)$-α-D-glucosidic bonds broken in the hydrolysis reactions (5)–(7). Also given in Table 4 are values of $\Delta_r X_m^\circ/N$ for the hydrolysis reaction (36) of a linear oligosaccharide G_n. In this case, the value of $\Delta_r H_m^\circ/N$ is based upon the results of Goldberg et al. [11] for the $\Delta_r H_m^\circ$'s for reaction (36). The value of $\Delta_r C_{p,m}^\circ/N$ for reaction (36) is based on the results of Briggner and Wadsö [10] for $C_{p,2,m}^\circ$ for the substances G_n ($n = 1$ to 4) and on the heat capacity of $H_2O(l)$ [13]. The value of $\Delta_r S_m^\circ/N$ for reaction (36) is the same estimate as used above for $\Delta_r S_m^\circ$ for reaction (37). Examination of Table 4 shows that there is a difference of 3.95 kJ mol^{-1} in the values of $\Delta_r H_m^\circ/N$ for reactions (5) and (36). This enthalpy difference drops to 2.44 kJ mol^{-1} in a similar comparison of the values of $\Delta_r H_m^\circ/N$ for reactions (6) and (36) and to 2.01 kJ mol^{-1} for reactions (7)–(36). These enthalpy differences all correspond to a destabilization of the cyclodextrin relative to that of the linear oligosaccharide.

The thermodynamic quantities obtained in this study (see Table 3) allow one to calculate the equilibrium constants for these reactions over a wide range of temperature. An examination of the temperature dependency of the equilibrium constants of reactions (23)–(25) shows that the position of equilibrium of these reactions is not substantially affected by changes in the temperature of reaction. Thus, if one wishes to synthesize these cyclodextrins in substantial yields from linear maltodextrins, it is crucial to remove the cyclodextrins as they are formed in the reaction mixture. Thus, Le Châtelier's principle can be used to move the reaction in the desired direction. Therefore, ligands such as octadecanoic acid, cyclohexanepropanamide-hexanoic acid, and glycyrrhizic acid have been utilized for the removal and concomitant purification of α, β, and γ-cyclodextrin, respectively, from reaction mixtures [15,16].

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Table 4
Values of the quantities $\Delta_r H_m^\circ/N$, $\Delta_r G_m^\circ/N$, $\Delta_r S_m^\circ/N$, and $\Delta_r C_{p,m}^\circ/N$ for the hydrolysis reactions (5)–(7) and (36)

No.	Reaction	$(\Delta_r H_m^\circ/N)$ /(kJ mol^{-1}) [a]	$(\Delta_r G_m^\circ/N)$ /(kJ mol^{-1})	$(\Delta_r S_m^\circ/N)$ /(J K^{-1} mol^{-1})	$(\Delta_r C_{p,m}^\circ/N)$ /(J K^{-1} mol^{-1})
5	α-cyclodextrin(aq) + 6 $H_2O(l)$ = 6 D-glucose(aq)	$-(8.48 \pm 0.2)$	-18.3 [b]	32.8 [b]	31 ± 3
6	β-cyclodextrin(aq) + 7 $H_2O(l)$ = 7 D-glucose(aq)	$-(6.97 \pm 0.14)$	-17.7 [b]	36.1 [b]	15 ± 8
7	γ-cyclodextrin(aq) + 8 $H_2O(l)$ = 8 D-glucose(aq)	$-(6.54 \pm 0.15)$	-18.0 [b]	38.5 [b]	11 ± 3
36	$G_n(aq) + (n-1)H_2O(l) = nG_1(aq)$	$-(4.53 \pm 0.04)$	-16.5 [b]	40 [b]	$-(4 \pm 4)$

[a] N is the number of $(1 \rightarrow 4)$-α-D-glucosidic bonds broken in each of these reactions.
[b] Based upon an estimated value of $\Delta_r S_m^\circ$ for reaction (37).

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