ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 322 (1999) 67–76

CARBOHYDRATE RESEARCH

Cyclomaltoheptaose (β-cyclodextrin) inclusion complex formation with chlorogenic acid: hydration enthalpy, the solvent entropy (hydrophobic) effect, and enthalpy–entropy compensation

Peter Irwin *, Janine Brouillette, Andrew Giampa [1], Kevin Hicks, Andrew Gehring, Shu-I Tu

US Department of Agriculture [2], ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Received 15 January 1999; accepted 28 June 1999

Abstract

Direct isothermal titration calorimetric measurement of ΔH and ΔS for the inclusion of chlorogenic acid (CA) by epichlorohydrin-polymerized cyclomaltoheptaose (β-cyclodextrin, β-CD$_n$), β-CD, and hydroxypropyl-β-CD (HP-β-CD) was performed at various temperatures ($T = 10$, 25, 40, and 55 °C) in 0.1 M Na phosphate buffer (pH 6.7). β-CD$_n$ and HP-β-CD, but not soluble β-CD, binding reactions with CA exhibited a significant variation in ΔH as a function of T ($\Delta C_p = -228 \pm 51$, -145 ± 32, and -10 ± 38 J mol^{-1} deg^{-1}, respectively). These CD–CA systems exhibited normal enthalpy–entropy compensation ($T\Delta S$ versus ΔH) behavior with a slope close to unity ($\alpha = 1.05 \pm 0.1$) and small intercept ($T\Delta S_0 = 18$ kJ mol^{-1}). Thus, as ΔG was fairly uniform (-17 ± 2 kJ mol^{-1}) across T, mutually compensating adjustments in solvent-associated perturbations in both ΔS and ΔH for binding occurred. Relatively large unfavorable changes in ΔS (e.g., ΔS_{solv} decreased with T) mainly occurred in the substituted β-CD–CA systems and were compensated for by commensurate changes in ΔH. This thermodynamically favorable enthalpic deficit (negative values increasing with T) was negatively correlated with T-dependent alterations in the number of water molecules bound by the β-CD$_n$–CA complexes. This interpretation agrees with previous work [*Carbohydr. Res.*, 282 (1996) 65–79], whereupon the convex curvature in the partitioning of CA by β-CD$_n$ as a function of T, implying a non-vanishing ΔC_p, was eliminated with low water activity. Published by Elsevier Science Ltd.

Keywords: Cyclomaltoheptaose; β-Cyclodextrin; β-Cyclodextrin polymer; Chlorogenic acid; Thermodynamics; Binding; Enthalpy–entropy compensation; Binding heat capacity change

1. Introduction

In certain plant-derived foods, such as juices, enzymatic (polyphenol oxidase, PPO) browning occurs due to the oxidation and subsequent condensation of naturally occurring phenolic compounds such as chlorogenic acid (CA) and results in an undesirable pigmentation of the product. The control of en-

* Corresponding author. Tel.: +1-215-233-6420; fax: +1-215-233-6406.

E-mail address: pirwin@arserrc.gov (P. Irwin)

[1] Summer student, Lehigh University, Bethlehem, PA, USA.

[2] Reference to brand name or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

Published by Elsevier Science Ltd.
PII: S0008-6215(99)00204-9

zymatic browning in fresh plant products is a problem for the food processing industry since the utilization of sulfites has been restricted by the Food and Drug Administration (FDA) [1]. Thus, the inhibition of enzymatic browning in minimally processed plant products offers a significant economic use for sulfite alternatives such as cyclodextrins (CDs) [2,3]. CD–CA inclusion complexes have been well characterized (stoichiometry, 1:1), and the inhibition of enzymatic browning in apple juice has been shown to be directly correlated with various CD–CA binding constants [4]. One commercially practical [4,5] type of CD for food application is the insoluble form, such as epichlorohydrin-polymerized β-CD (β-CD_n) [6–8], which can be removed from the treated juice, regenerated and reused numerous times.

Various mechanisms have been proposed as the driving force for CD inclusion complex formation [9–11] with manifold guest molecules. Some of these are related to the effect of the solvent, usually water, on the complex [3,9]. In previous work [3,5], the partitioning of CA by soluble and polymerized β-CD was studied by characterizing the dependence of the apparent partition coefficient, or binding constant (K), on water activity (a_{H_2O}). These experiments were performed to help test the involvement of a 'phenomenologically significant' [12] species of water believed to bestow stability [13,14] on CD inclusion complexes. It was estimated that the stoichiometric coefficient for water (z), whereupon

$$K = K_0 a_{H_2O}^{z}$$

associated with the formation of β-CD_n–CA inclusion complexes varied from ca. 5 (7 °C) to 7 (42 °C), while the same parameter for soluble β-CD was relatively invariant [3,5] with temperature (T). This hypothesis was supported by ^{1}H NMR spin–lattice relaxation data [3]. From a pragmatic perspective [5], such changes in the CD–guest water structure with T have commercial consequences for the utilization of β-CD_n, since food systems are extremely heterogeneous from the standpoint of a_{H_2O} and solute concentration and, thus, could alter the binding potential of the matrix.

Thus, many [3–5,9–11,13–17] studies indicate that water plays a significant role in CD inclusion complex stability. Since solvation of the complex (favorable ΔH of hydration [18]) and desolvation of the binding site (the 'hydrophobic effect') appear to play an important part in β-CD systems, we were interested in determining if heat-capacity changes for binding ($\Delta C_p = \partial \Delta H / \partial T$) were evident in CD–CA interactions. ΔC_p is of fundamental interest since it is related to both hydrophobic, or 'solvent entropy effect', interactions and other hydration enthalpic changes with T [18–25]. Determination of ΔC_p is best performed by calorimetric techniques (i.e., direct detection of ΔH at various temperatures), such as isothermal titration calorimetry (ITC) [26]. Without calorimetric techniques such as ITC, ΔH can only be accurately estimated [3,4] by assuming that the T dependence of K follows the relationship

$$K = \exp(-\Delta H/RT)\exp(\Delta S/R)$$

Obviously, this equation assumes that ΔH has no T dependence. This assumption did not seem problematic with the β-CD–CA system [3,4] since the apparent K appeared to follow the relation above. However, the β-CD_n–CA interaction [4,5] displayed an unusual T dependency whereupon K increased substantially from 7 to 28 °C and decreased between 28 and 56 °C. Such convex curvature in K–T plots indicated that ΔH varied substantially with T. Thus, our present effort extends [3,5] the study of the β-CD–CA inclusion complex system by investigating the thermochemistry of the T dependence of CA partitioning by various β-CDs using ITC.

2. Results and discussion

Calorimetry.—Two sets of raw (A) and processed (B) data are shown in Fig. 1 for an automated sequence of 30 CA injections into an ITC cell containing β-CD_n suspended in 0.1 M Na phosphate buffer (see Fig. 1 caption for details). In all work presented herein, two sets (each replicated thrice) of ITC experiments were performed so that ΔH could be measured independently from the other experimental parameters (ΔG and ΔS) of interest. ΔH can be determined this way only when the host

(CD) molecules saturate the guest (CA) species, resulting in titration peaks that were essentially constant (Fig. 1(A), inset). A normal titration experiment (e.g., CA saturating) can then be performed and integrated, and lines of best fit can be iteratively solved to deconvolve (see Section 4, [27]) the other thermodynamic parameters (ΔG and ΔS). Such manipulations of the data in Fig. 1(A) show that the best-fit curves (Fig. 1(B), $\Delta H = -19 \pm 1$ kJ mol^{-1} and $\Delta G = -17 \pm 1$ kJ mol^{-1}; $\pm$ asymptotic standard error, ε [28]) pass closely through the experimental points even though the data were transformed using the derivative convention,

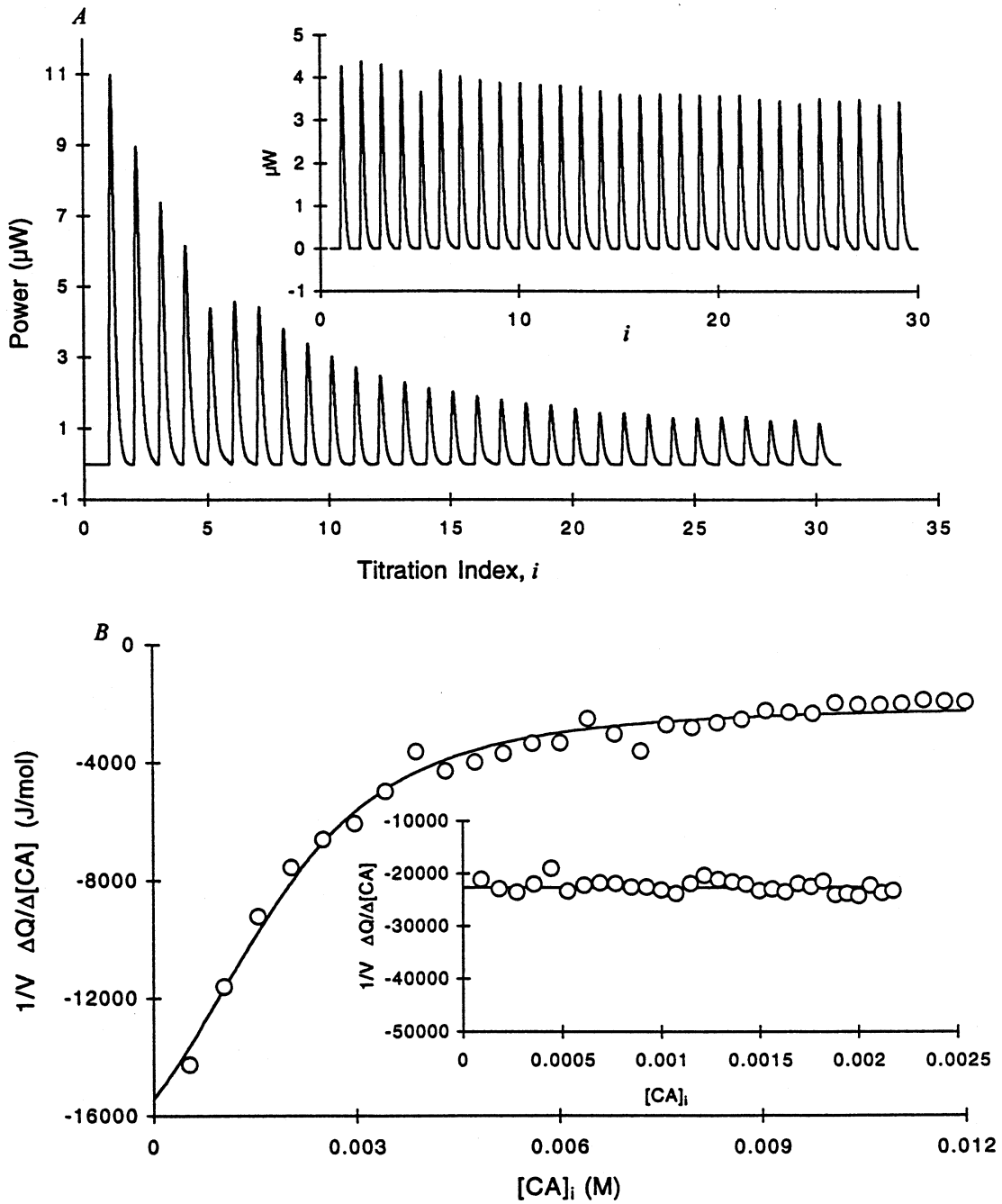

Fig. 1. (A) Raw data ($T = 25$ °C) obtained for 30 injections, each of 8 μL, of CA solution (50 mM in 0.1 M Na phosphate buffer) into a sample cell containing 760 μL of the β-CD$_n$ slurry (17.2 mg wet weight); inset: 10 mM CA, 512 mg of (wet) β-CD$_n$ slurry (864 μL total volume). (B) Plots of processed data (corresponding to raw data in Fig. 1(A)) in the derivative format. The data points are experimental and the solid lines correspond to the best-fit curve obtained by deconvolution (see Section 4); for these fits $\Delta H = -19$ kJ mol^{-1}, $\Delta G = -17$ kJ mol^{-1}, and $\Delta S = -7$ J mol^{-1} deg^{-1}.

Table 1
Thermodynamic parameters derived from isothermal titration calorimetry (ITC) concerning the binding of CA by β-CD$_n$, HP-β-CD, and soluble β-CD at various temperatures

T (°C)	ΔG [a] (kJ mol^{-1})			ΔH (kJ mol^{-1})			ΔS (J mol^{-1} deg^{-1})		
	β-CD$_n$	HP-β-CD	β-CD	β-CD$_n$	HP-β-CD	β-CD	β-CD$_n$	HP-β-CD	β-CD
10	-17.06 ± 0.76	-15.95 ± 0.65	-19.62 ± 0.58	-12.95 ± 0.21	-13.47 ± 0.19	-14.25 ± 0.07	14.49 ± 3.11	8.77 ± 2.94	18.98 ± 2.30
25	-17.51 ± 0.24	-16.23 ± 0.61	-18.06 ± 0.15	-19.39 ± 0.43	-17.68 ± 1.60	-12.65 ± 0.25	-6.32 ± 2.25	-4.86 ± 5.56	18.15 ± 0.54
40	-18.16 ± 0.56	-17.75 ± 1.47	-17.98 ± 0.22	-21.74 ± 0.27	-18.38 ± 1.18	-12.74 ± 0.88	-11.44 ± 2.59	-2.02 ± 0.93	16.73 ± 3.03
55	-17.60 ± 0.43	-14.75 ± 0.20	-16.31 ± 0.39	-23.59 ± 1.01	-20.47 ± 0.39	-14.71 ± 0.37	-18.51 ± 3.96	-17.44 ± 1.70	4.88 ± 2.33
t [b]	1.35	1.59	0.56	1.39	2.48	1.13	7.67	5.10	4.95
				ΔC_p [c] (J mol^{-1} deg^{-1})					
				-228 ± 51	-145 ± 32	-10 ± 38			

[a] Average of three experiments ± standard deviation.
[b] $t_{0.05}$ {error sums of square/error degrees of freedom}$^{1/2}$.
[c] Slope of ΔH with respect to $T \pm$ error of the slope.

which tends to magnify any experimental errors [27]. Identical experiments to the ones depicted in Fig. 1 were also performed using β-CD (unsubstituted) and HP-β-CD (as a substituted β-CD control) and are presented in Table 1 ($\pm$ standard deviations for all observations).

Temperature dependence of ΔH: hydration enthalpy.—Substituted β-CD–CA complexes (β-CD$_n$ and HP-β-CD; Table 1, Fig. 2) displayed relatively large changes in ΔH with T (ΔH of ca. -11 and -7 kJ mol^{-1}, respectively), which resulted in substantial ΔC_ps (-228 ± 51 and -145 ± 32 J mol^{-1} deg^{-1}) whereupon

$$\Delta H = \Delta H_0 + \Delta C_p T$$

The results in Fig. 2 also revealed that the ΔH for CD–CA complex formation converged as T approached the freezing point of water ($\Delta H_0 = -12 \pm 2$, -13 ± 1, and -13 ± 1 kJ mol^{-1}, respectively, for β-CD$_n$–, HP-β-CD–, and β-CD–CA; $\pm$ standard error of the intercept). The calorimetric determination of ΔH (-13.59 ± 1.13 kJ mol^{-1}) for the formation of the unsubstituted β-CD–CA complex was reasonably close to this same parameter (-11 kJ mol^{-1}) obtained from indirect [3,4] methods, and its T dependence yielded only a small ΔC_p (-10 ± 38 kJ mol^{-1}; Table 1, Fig. 2). It should be noted that, contrary to our findings, non-vanishing but T-independent ΔC_ps (-333 ± 4 J mol^{-1} deg^{-1}; $\Delta G = -16 \pm 0.1$ kJ mol^{-1}) have been observed for unsubstituted β-CD–cyclohexanol [26] complex formation. This result indicates that thermodynamic properties can vary with the guest species, and therefore, ΔC_p is modulated by manifold interactions between both host and guest.

A major source of large negative enthalpy changes with T are intermolecular hydrogen bonds and van der Waals interactions. These forces, when combined with solvent-mediated hydrogen bonds, can generate favorable (negative) ΔHs and counter the unfavorable (also negative, Table 1) ΔS contributions to ΔG at higher temperatures [18]. The inset in Fig. 2 shows calculated ΔHs (from the T dependence discussed previously) for both soluble and polymerized β-CD–CA systems plotted against measured values [3,5] of waters of hydration (z) whereupon

$$\beta\text{-CD}\text{–}[H_2O]_x + \text{CA}\text{–}[H_2O]_y + zH_2O$$
$$\leftrightarrow \beta\text{-CD–CA}\text{–}[H_2O]_{x'+y'+z} \quad (x \geq x' \text{ and } y \geq y')$$

In the β-CD$_n$–CA system there was a significant negative correlation ($r = -0.94$) between ΔH and these waters of hydration, which are presumably structural [3,5] inasmuch as they can link the host to the guest species through hydrogen bonds resulting in a favorable hydration enthalpy ΔH (e.g., increasingly negative with T). Structural water of this sort has been shown, using high-resolution X-ray crystallography and ITC, to play an important role in stabilizing certain antibody–antigen (Ab–Ag) complexes [18]. Contrariwise, both ΔH (-13 ± 0.1) and z (6.2 ± 0.1) were reasonably constant with T in the β-CD–CA system.

Temperature dependence of ΔS: the solvent entropy effect.—There are a number of contributors (subscript i, below) to the entropy change (ΔS) in any system (e.g., enzyme–inhibitor, antibody–antigen or CD–guest), that involves binding [18,23]: a solvent (ΔS_{solv}) term, a host–guest conformational (spatial) term, as well as a component related to the loss of translational–rotational (dynamic) degrees of freedom,

$$\Delta S = \sum_i \Delta S_i \propto \int \left(\sum_i \frac{\Delta C_{p,i}}{T} \right) \mathrm{d}T$$

Assuming that ΔS_{solv} at T_s (112 °C [25]) is vanishingly small and ΔC_p is dominated by the solvent's effect on ΔS [23,25] then,

$$\Delta S_{solv} = \Delta C_p \log_e T/T_s$$

Obviously, the latter is an unrealistic assumption since ΔC_p is linked, by definition, to both enthropic and enthalpic changes. However, we have used this expression to estimate the solvent (ΔS_{solv}, Fig. 3) and non-solvent (Fig. 3, inset) contributions to ΔS at various temperatures. The hydrophobic (solvent entropy) effect, which "drives the association of nonpolar

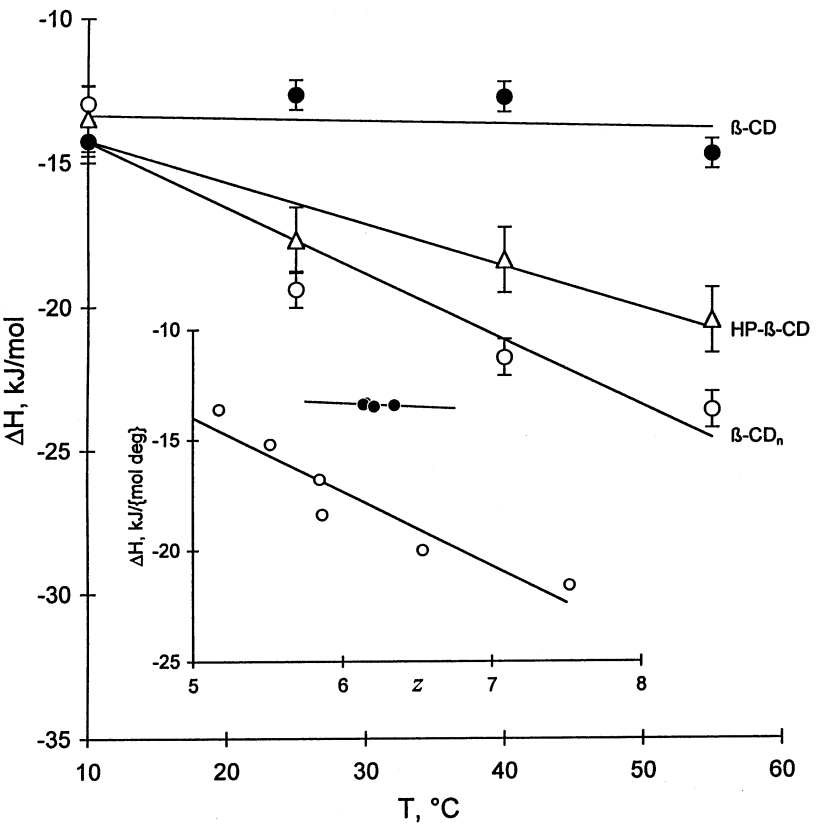

Fig. 2. Dependency of the ΔH of CD–CA complex formation on temperature for β-CD (closed circles), HP-β-CD (open triangles), and β-CD$_n$ (open circles). Data are provided with error bars, each representing the total experimental error ({error sums of square/error degrees of freedom}$^{1/2}$). The slopes of each line represent $\Delta C_p = \partial \Delta H/\partial T$ and are provided in Table 1. Inset: relationship of estimated ΔHs for β-CD$_n$–CA (open circles) and β-CD·CA (closed circles) to the hydration stoichiometric coefficient, z, associated with CD complex formation [3,5].

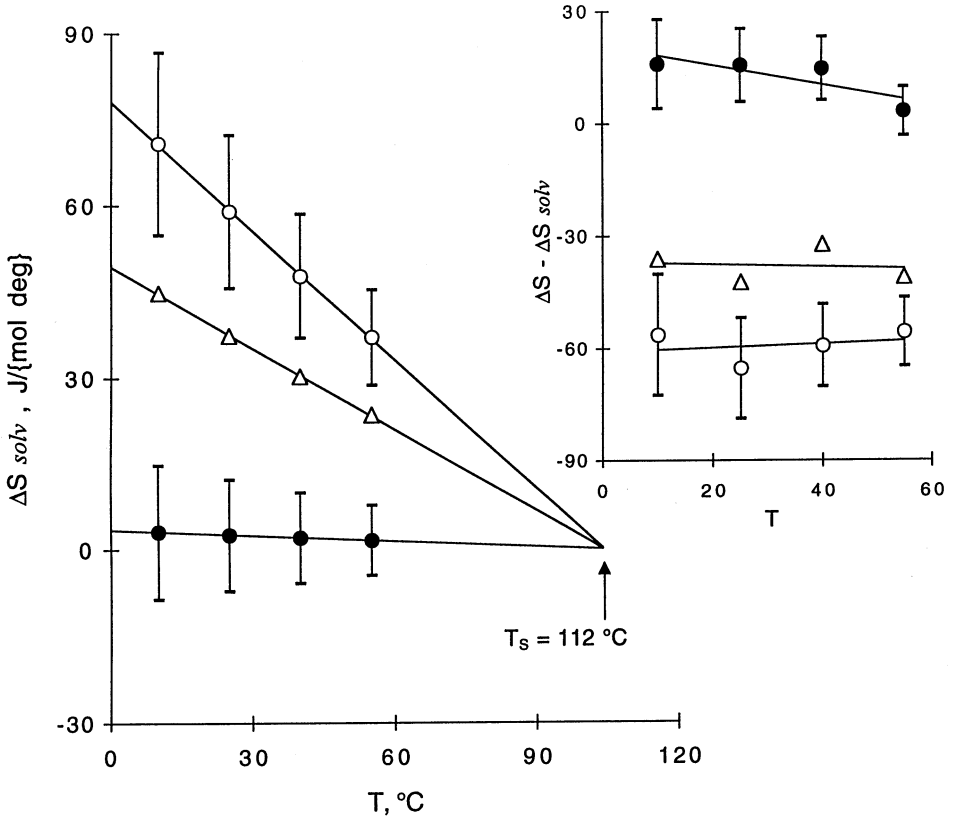

Fig. 3. Estimation of ΔS_{solv} at various temperatures for β-CD–CA (closed circles), HP-β-CD–CA (open triangles), and β-CD$_n$–CA (open circles); T_s (112 °C) is assumed to be the temperature at which ΔS_{solv} approaches 0 [25]. Inset: nonsolvent-related component of ΔS for binding ($\Delta S - \Delta S_{solv}$) at different T values for the various CD–CA complexes. Error bars ($\pm\sigma$, propagation of errors method [32]) are provided for β-CD$_n$ and β-CD (the extrema) in order to show the computational uncertainty.

surfaces of molecules by excluding H_2O from the interface" [21], is believed to contribute to ΔS [19–25]. If this is true, the dependence of ΔS on T (Table 1; associated mainly with the substituted β-CD–CA complexes) is due to hydrophobic interactions as evidenced by a flat T dependence of ΔS when ΔS_{solv} was removed (Fig. 3, inset). This entropic gain due to solvent release from the binding site at lower Ts seems to be a "common feature of biological systems by which they overcome the high energy cost" of creating apparent order out of chaos [23]. However, the overall negative ΔS at higher temperatures (β-CD$_n$– and HP-β-CD–CA, Table 1) suggests that the decrease in spatial and dynamic entropies upon complex formation (negative $\Delta S - \Delta S_{solv}$; Fig. 3, inset) is not compensated for by an increase in ΔS_{solv}. Thus, the substituted β-CD–guest interactions are enthalpically driven and thermodynamically linked to changes in concentration of structural waters of hydration [18].

Entropy–enthalpy compensation.—Enthalpy–entropy compensation [12] is a term utilized to describe the behavior of ΔH and ΔS for a series of similar reactions driven by changes in solvation (e.g., hydration enthalpies and the solvent entropy effect, Fig. 2, inset and Fig. 3) and is an ubiquitous phenomenon in many systems (Fig. 4). This compensatory behavior is considered to be "extra-thermodynamic" [12] and not an obvious consequence of thermodynamic law, since ΔH and ΔS are both related to ΔC_p,

$$\Delta H = \Delta H_{T_0} + \int_{T_0}^{T} \Delta C_p \mathrm{d}T \text{ and}$$

$$\Delta S = \Delta S_{T_0} + \int_{T_0}^{T} \frac{\Delta C_p}{T} \mathrm{d}T$$

The above equations show that these thermodynamic quantities are only linear with respect to each other as ΔC_p vanishes. In general, the linear behavior of $T\Delta S$ with ΔH is a consequence of a non-zero ΔC_p and is not due to

relatively small changes in ΔG with T. Thus, the extra-thermodynamic compensation behavior described herein, and elsewhere [3–5,12–14,17], is indicated only when a linear relationship exists between $T\Delta S$–ΔH pairs and ΔC_ps are non-zero. Typically, a series of ΔH–ΔS pairs for binding reactions is created by altering the solvent [3,5], changing the pH [4], or by working with different forms [13,14] of the interacting species. However such ΔH–ΔS pairs are created, they produce only one linear relationship for each class of chemical interaction. For instance, in numerous biological systems, increasingly unfavorable ΔSs with T (in our system dominated by $\Delta\Delta S_{solv}$, Fig. 3) are compensated for by equivalent changes in ΔH (Fig. 4: β-CD–, HP-β-CD–, β-CD$_n$–CA, and various Ab–Ag [19,22] data) and result in ΔG being relatively invariant (e.g., for β-CD–CA complexes, reported herein, $\Delta G = -17 \pm 2$ kJ mol^{-1}; Table 1). Thus, disparate systems have a slope ($\alpha = 0.99 \pm 0.03$ [anti-lysozyme Ab] and 0.97 ± 0.02 [anti-hapten Ab]; the Ab–hapten system is unusual in that ΔC_p is positive [1292 ± 107 J mol^{-1} deg^{-1}]) similar to that for β-CD–guest complexes ($\alpha = 1.05 \pm 0.1$; for β-CD–cyclohexanol [26] $\alpha = 0.95 \pm 0.02$) but widely different intercepts ($T\Delta S_0 = 45 \pm 2$, 23 ± 1, and 18 ± 2 kJ mol^{-1}, respectively; β-CD–cyclohexanol $T\Delta S_0 = 15.96 \pm 0.01$). $T\Delta S_0$ has been interpreted [13,14] as being related to the solvent entropy effect (relative desolvation of the binding site). However, these (Fig. 3) and other [18,22] data show that, while the solvent entropy effect does appear to modulate the T dependence of ΔS, the overall magnitude of entropic changes is clearly dominated by other factors such as decreases in translational–rotational and conformational entropies linked with complex formation. It is interesting (Fig. 4), and perhaps significant, that an oligosaccharide binding to a dextran-specific monoclonal Ab [19] results in a $T\Delta S_0$ (23 ± 1 kJ mol^{-1}) much closer to ours (18 ± 2 kJ mol^{-1}) than the same term expressed for an Ab–protein system ($T\Delta S_0 = 45 \pm 2$ kJ mol^{-1}). We suspect that differences in $T\Delta S_0$ between binding systems are related to the total area of the complex interface.

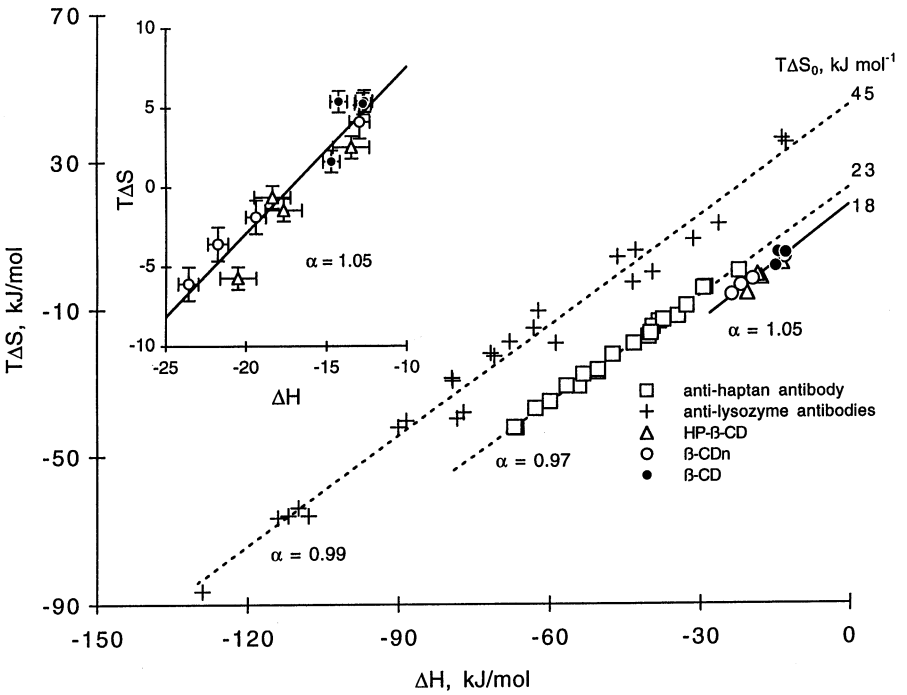

Fig. 4. Enthalpy–entropy compensation plot for β-CD$_n$–CA (open circles), HP-β-CD–CA (open triangles), and β-CD–CA (closed circles) thermodynamic parameters. As a comparison we have provided values for other interacting systems (+ symbols and open squares for the Ab–lysozyme [22] and ·hapten [19] systems, respectively) which display similar behavior with respect to α (parallel lines of best fit; $T\partial\Delta S/\partial\Delta H = \alpha \sim 1$) yet have greater intercepts ($T\Delta S_0$). For numerous other [14] β-CD–guest complexes (data not shown) we calculated that $\alpha = 0.84 \pm 0.06$ and $T\Delta S_0 = 14.49 \pm 1.37$ kJ mol^{-1}. Inset: enthalpy–entropy compensation plot for the various CD–CD complexes; error bars represent the standard deviations for each mean value of $T\Delta S$ and ΔH.

3. Conclusions

We have performed direct (ITC) measurement of ΔH for the inclusion of CA by β-CD$_n$, β-CD, and HP-β-CD at 10, 25, 40, and 55 °C in 0.1 M Na phosphate buffer (pH 6.7). We found that β-CD$_n$ and HP-β-CD (not β-CD) complex formation with CA resulted in a significant ΔC_p (-228 ± 51 and -145 ± 32 J mol^{-1} deg^{-1}, respectively). While values for ΔG were fairly uniform (-17 ± 2 kJ mol^{-1}) across T, large changes in ΔS_{solv} appeared to occur and argue that the β-CD$_n$ system underwent greater desolvation at its binding site than the β-CD–CA system. This interpretation agrees with previous work [5], which indicated that the convex curvature in K–T plots (hypothesized to be due to nonvanishing ΔC_ps) was eliminated at lower a_{H_2O}s. Thus, relatively large changes in solvent-associated perturbations in ΔS occurred and were compensated for by commensurate changes in ΔH. This favorable enthalpic deficit was, in turn, associated with T-dependent alterations in the number of bound water molecules, which appear to play a role in stabilizing the host–guest complex.

4. Experimental

General.—β-CD and β-CD$_n$ (40–60 mesh, Lot # 92-1) were donated by Cerestar USA (1100 Indianapolis Blvd., Hammond, IN 46320-1094, USA). CA, 3-*O*-(3,4-dihydroxycinnamoyl)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, was purchased from Sigma Chemical Company. HP-β-CD (M_W 1380, ca. 4.2 substitutions per CD) was obtained from the Aldrich Chemical Company. All water used in these experiments was processed from a deionized–distilled source to have a low conductivity (ca. 10^{-7} Ω^{-1}). The buffer used was equimolar mono- and dibasic Na phosphate (100 mM total phosphate) at a pH of ca. 6.7. All experiments were performed at 10, 25, 40 or 55 °C.

Experiments were executed to determine the proportionality between wet and dry weight (ca. 3.74) for the β-CD$_n$ samples. Between experiments β-CD$_n$ (ca. ten parts buffer to one part β-CD$_n$) was stored at 4 °C under vacuum. For each binding experiment ca. 1 mL of β-CD$_n$ matrix was washed with 5 mL of buffer. Excess fluid was removed by filtering the slurry through an acid-washed, coarse, sintered glass filter apparatus. β-CD$_n$ samples were weighed out and then loaded into the 1-mL stainless steel calorimeter cell; the volume was brought to a total of ca. 0.7–0.9 mL with buffer. All samples (β-CD$_n$, β-CD, and HP-β-CD) were degassed ca. 10 min immediately prior to ITC examination.

Calorimetry.—Studies were performed on a Calorimetry Sciences Corporation (CSC, PO Box 799, Provo, UT 84603-0799) 4200 ITC. The ITC was calibrated at each T used in the study: 30 injections of 8 mL with ca. 6 mM $BaCl_2$ (dried thoroughly prior to utilization) into 0.76 mL of 180 mM 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) [29] in water. For all experiments data were collected at a rate of one point every 4.5 s (1800 s between injections). All experiments reported herein were replicated thrice for each thermodynamic component (e.g., one set of three experiments for ΔH; one set of three for ΔG). For instance, ΔH was determined by titrating with CA (30 injections of 8 mL each with 10 mM CA) into a solution (or slurry for β-CD$_n$) containing 0.76 mL of a buffer–CD mixture; the starting CD concentrations were made such that the final molar ratio of [CD] to [CA] was large enough (ca. 20–50:1) to result in essentially constant titration peaks (Fig. 1(A), inset). Three sets of ΔG experiments were also performed (each using 30 titrations of 8 mL of 50 mM CA) such that the final [CA]:[CD] ratio was ca. 2–3. Fig. 1(A) shows the result for both types of experiment with CA being titrated into a slurry of β-CD$_n$.

Deconvolution of thermodynamic parameters.—Raw data (dQ/dt, in units of μW, versus time) from titrations (Fig. 1(A)) were integrated, and the resultant corrected (i.e., integrated control peaks, $\Delta Q_{control}$, were subtracted) areas, ΔQ, were iteratively fit [30] to an equation (derived by Wiseman et al. [27]) describing the derivative ($V^{-1} \times \Delta Q \times \Delta[CA]^{-1}$; V = volume of cell after each ith injection; $\Delta[CA] = [CA]_i - [CA]_{i-1}$) of heat evolved (positive peaks, exothermic) or ab-

sorbed (negative peaks, endothermic) with respect to titrant ([CA]) and CD ([CD]) concentration after each injection whereupon,

$$\frac{1}{V}\frac{\Delta Q}{\Delta[\mathrm{CA}]} =$$

$$\frac{\Delta H}{2}\left(1+\frac{K[\mathrm{CD}]n-[\mathrm{CA}]K-1}{K\sqrt{\frac{\{1+[\mathrm{CA}]nK+[\mathrm{CD}]K\}^2}{K^2}-4n[\mathrm{CA}][\mathrm{CD}]}}\right)+\Delta H^*$$

Control experiments consisted of identical titrations but with the buffer containing no CD. Typically $\Delta Q_{\mathrm{control}}$ varied in a linear fashion from -100 to -400 mJ with the titration index, *i*. In the above equation, K is the binding constant and ΔH is the enthalpy change in the system due to binding. ΔH^* is an additional term we have added to correct for any constant background process not accounted for by subtracting out $\Delta Q_{\mathrm{control}}$. We have found [31] that such a background signal can be substantial in certain heterogeneous systems (such as in the β-CD$_n$ system). ΔH^* was only a substantial component in the β-CD$_n$ experiments (ca. -2 to -5 kJ mol^{-1}, depending on T) and appeared to be related to the heat of dilution of CA within the polymer's matrix (whereupon $\Delta C_p = -57 \pm 18$ J mol^{-1} deg^{-1}). The initial value of ΔH used in the iterative solving of $\{V^{-1} \times \Delta Q \times \Delta[\mathrm{CA}]^{-1}\}$ as a function of [CA] and [CD] was calculated from experiments, described above, with saturating levels of CD (Fig. 1(B), inset). A correction

$$\Delta H = \{\Delta H_{\mathrm{obs}} + 0.27\} - \{0.9675\Delta H^*\}$$

was continually made on ΔH during iterative solving based on the multiple linear regression analysis of the effects of a constant background on known values of ΔH; this correction altered ΔH, at most, by 10%. Knowing K and ΔH from the above relationships, ΔG and ΔS were obtained from

$$\Delta G = -RT \log_e K$$

$$\Delta S = (\Delta H - \Delta G)/T$$

We have tested our computational technique, a spreadsheet Gauss–Newton (GN) algorithm developed in our laboratory [28], against the Levenberg–Marquart (LM; [30]) method on the same data (titrations of 2′CMP into RNaseA at 38 °C) using [27] identical mathematical models, except that ours incorporated the ΔH^* baseline correction term. The LM curve-fitting procedure yielded: $\Delta H = -57$ kJ mol^{-1}, $\Delta S = -93$ J mol^{-1} deg^{-1} [27]; the GN [28] procedure gave: $\Delta H = -56 \pm 0.3$ kJ mol^{-1}, $\Delta H^* = -0.4 \pm 0.2$ kJ mol^{-1}, $\Delta S = -87 \pm 3$ J mol^{-1} deg^{-1} ($\pm\varepsilon$, asymptotic standard error). All results were in good agreement and argue that our ΔH^*-corrected derivative GN method provides reasonable thermodynamic parameters for ITC data.

Acknowledgements

We thank Drs D. Downing, A. Hedges and R. Friedman of Cerestar USA for the donation of various CD products.

Nomenclature

K	binding, formation or association constant; partition coefficient
ΔG	Gibb's function $= \Delta H - T\Delta S$
ΔH	enthalpy change
n	molar ratio of available: all binding sites; an apparent, or effective, stoichiometric coefficient
z	true stoichiometric coefficient (as in the number of moles of water bound per mole of CD–CA complex); NMR studies with soluble β-CD [4] and CA show this value to be approximately one
ΔC_p	isobaric heat-capacity change; $\partial\Delta H/\partial T$
ΔH^*	background process which might be related to the heat of dilution of CA within the β-CD$_n$'s polymer matrix
ΔQ	heat changes from integration of calorimetric titration endo- or exotherms
ΔS	entropy change; $\Delta S = \Sigma_i \Delta S_i$ ($i =$ solvation, rotational/rotational, and conformational components of ΔS

α	slope of the $T\Delta S$ vs ΔH plot
$T\Delta S_0$	intercept (at $\Delta H = 0$) of the $T\Delta S$ vs ΔH plot
T	temperature in units of Kelvin (K) or Centigrade (°C)
a_{H_2O}	water activity
CA	3-*O*-(3,4-dihydroxycinnamoyl)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid (trans form [4])
β-CD$_n$	polymerized β-CD (40–60 mesh)
HP-β-CD	hydroxypropyl β-CD (M_W 1380, ca. 4.2 substitutions per CD)
Ab	antibody
Ag	antigen

References

[1] G. Sapers, *J. Food Technol.*, 54 (1991) 75–84.
[2] K. Hicks, G. Sapers, P. Seib, US Pat. 4,975,293 (1990).
[3] P. Irwin, J. Brouillette, K. Hicks, *Carbohydr. Res.*, 269 (1995) 201–216.
[4] P. Irwin, P. Pfeffer, L. Doner, G. Sapers, J. Brewster, G. Nagahashi, K. Hicks, *Carbohydr. Res.*, 256 (1994) 13–27.
[5] P. Irwin, G. King, K. Hicks, *Carbohydr. Res.*, 282 (1996) 65–79.
[6] P. Shaw, B. Buslig, *J. Agric. Food Chem.*, 34 (1986) 837–840.
[7] G. Crini, C. Cosentino, S. Bertini, A. Naggi, G. Torri, C. Vecchi, L. Janus, M. Morcellet, *Carbohydr. Res.*, 308 (1998) 37–45.
[8] R. Friedman, A. Hedges, F. Black, D. Gottneid, *Carbohydr. Res.*, 192 (1989) 283–289.
[9] K. Connors, D. Pendergast, *J. Am. Chem. Soc.*, 106 (1984) 7607–7614.
[10] W. Gerasimowicz, J. Wojcik, *Bioorg. Chem.*, 11 (1982) 420–427.
[11] J. Szejtli, *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988, pp. 79–185.
[12] R. Lumry, S. Rajender, *Biopolymers*, 9 (1970) 1125–1227.
[13] Y. Inoue, Y. Yiu, L.-H. Tong, B.-J. Shen, D.-S. Jin, *J. Am. Chem. Soc.*, 115 (1993) 10637–10644.
[14] Y. Inoue, T. Hakushi, Y. Liu, L.-H. Tong, B.-J. Shen, D.-S. Jin, *J. Am. Chem. Soc.*, 115 (1993) 475–481.
[15] T. Furuta, H. Yoshii, A. Miyamoto, A. Yasunishi, H. Hirano, *Supramol. Chem.*, 1 (1993) 321–325.
[16] H. Yoshii, T. Furuta, A. Yasunishi, H. Hirano, *J. Biochem.*, 115 (1994) 1035–1037.
[17] R. Clarke, J. Coates, S. Lincoln, *Adv. Carbohydr. Chem. Biochem.*, 46 (1988) 205–249.
[18] T. Bhat, G. Bentley, G. Boulot, M. Greene, D. Tello, W. Dall'Acqua, H. Souchon, F. Schwarz, R. Mariuzza, R. Poljak, *Proc. Natl. Acad. Sci. USA*, 91 (1994) 1089–1093.
[19] R. Zidovetzki, Y. Blatt, G. Schepers, I. Pecht, *Mol. Immunol.*, 25 (1988) 379–383.
[20] C. Raman, M. Allen, B. Nall, *Biochemistry*, 34 (1995) 5831–5838.
[21] H. Torigoe, T. Nakayama, M. Imazato, I. Shimada, Y. Arata, A. Sarai, *J. Biol. Chem.*, 270 (1995) 22218–22222.
[22] F. Schwarz, D. Tello, F. Goldbaum, R. Mariuzza, R. Poljak, *Eur. J. Biochem.*, 228 (1995) 388–394.
[23] K. Murphy, E. Freire, Y. Paterson, *Proteins Struct. Funct. Genet.*, 21 (1995) 83–90.
[24] I. Jelesarov, L. Leder, H. Bosshard, *Methods: Companion Methods Enzymol.*, 9 (1996) 533–541.
[25] K. Tsumoto, K. Ogasahara, Y. Ueda, K. Watanabe, K. Yutani, I. Kumagai, *J. Biol. Chem.*, 271 (1996) 32612–32616.
[26] H. Naghibi, A. Tamura, J. Sturtevant, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 5597–5599.
[27] T. Wiseman, S. Williston, J. Brandts, L.-N. Lin, *Anal. Biochem.*, 179 (1989) 131–137.
[28] P. Irwin, W. Damert, L. Doner, *Concepts Magn. Reson.*, 6 (1994) 57–67.
[29] L.-E. Briggner, I. Wadsö, *J. Biochem. Biophys. Methods*, 22 (1991) 101–118.
[30] N. Draper, H. Smith, *Applied Regression Analysis*, second ed., Wiley, New York, 1981, pp. 462–465.
[31] P. Irwin, J. Brouillette, S. Osman, K. Hicks, *Carbohydr. Res.*, 311 (1998) 37–49.
[32] P. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, 1969, pp. 56–65.