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Cyclomaltoheptaose (β-cyclodextrin) and hydroxyethyl-substituted β-cyclodextrin inclusion complex formation with chlorogenic acid: solvent effects on inclusion complex stability

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Abstract

The inclusion complexes of cyclomaltoheptaose (β-CD) and β-CD's 50% hydroxyethyl-substituted derivative (HE-β-CD) with chlorogenic acid (CA) were studied with regard to temperature and water activity ($a_{H_2O} \approx$ mole fraction $= X_{H_2O} = 0.8$–0.99; 0.1 M Na phosphate buffer) utilizing first-derivative spectrophotometric analyses of bathochromic shifts ($\Delta\lambda$) in CA's UV absorbance as a function of variable [CD]. From the dependence of the apparent stability constant, K, on X_{H_2O} ($K = K^{\ddagger}X^{z}_{H_2O}$) we estimated that the β-CD · CA complex's apparent stoichiometric coefficient, z, for water was ca. 7 ± 1 ($K^{\ddagger} = 1032 \pm 54$ M^{-1}); this value agrees with recently published literature concerning the minimum number of waters needed to stabilize a similar β-CD adduct. However, we determined that z was significantly lower (4 ± 0.3; $K^{\ddagger} = 809 \pm 31$ M^{-1}) for the HE-β-CD · CA complex. These results argue that a unique species of bound water is involved in β-CD · CA stability since a 50% substitution resulted in an equivalent loss in z as well as a substantial decrease in $K^{\ddagger}$. This hypothesis was supported by NMR inversion recovery experiments whereupon the most significant perturbation to spin-lattice relaxation ($\Delta T_1 = T_{1_{\beta\text{-CD}}} - T_{1_{\beta\text{-CD}\cdot\text{CA}}}$) was associated with β-CD's ^{1}H at position 3 (H-3; $\Delta T_1 = 585$ ms). Small ΔT_1s were also observed for H-2 (160 ms) and H-6,6′ (83 ms). β-CD's H-3 ΔT_1s were dependent not only upon the adduct's concentration but also diminished at a high ionic strength. These data indicate that ΔT_1 was related to changes in [D_2O] at or near β-CD's hydroxyl groups and that these D_2O molecules were bound with a relatively long residence time. Thermochemical measurements of

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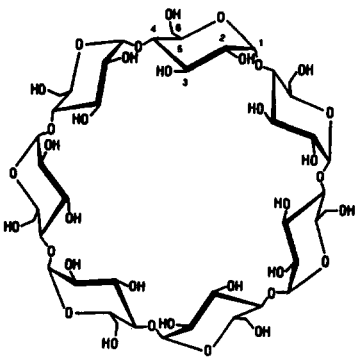
ΔH and ΔS at various X_{H_2O}s display typically linear enthalpy–entropy compensation (ΔH–ΔS) relationships but with a slope ($T_c = \partial\Delta H/\partial\Delta S = 272$ K) significantly less than standard aqueous thermodynamic measurements ($T_c = 305$ K) of a similar system. This unequivocal X_{H_2O} effect on T_c argues that the chemical part process of CD · guest adduct formation involves changes in relative solvation, presumably desolvation, of β-CD's binding site. This interpretation was supported by the dependency of $\Delta\lambda_{max}$ on CD binding site dimension and $X_{H_2O}^{MeOH}$.

Keywords: Cyclomaltoheptaose (β-cyclodextrin); Chlorogenic acid; Inclusion complex; Stability

1. Introduction

In previous work [1], the binding geometry, stoichiometry (1 : 1), and thermodynamic behavior of various cyclomalto–oligosaccharide (cyclodextrins or CDs; β-CD, **1**) inclusion complexes with chlorogenic acid (CA, **2**) [3-*O*-(3,4-dihydroxycinnamoyl)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid], a major substrate for polyphenol oxidase (PPO), were characterized. The mechanism of CD inhibition of enzymatic browning in apple juice was also shown to be directly related to the apparent stability constant (K) for the binding of **2** rather than the sequestration of Cu^{2+} from the active site of PPO. The amelioration of enzymatic browning in fresh fruit and vegetable products proffers a significant economic use [2] for **1**, and its derivatives, since the utilization of sulfites, the most efficacious group of browning inhibitors, has been restricted by the U.S. Food and Drug Administration (FDA) [3]. Basic knowledge gained in this area will be useful in the prediction and identification of natural antibrowning oligo- and poly-saccharides which are likely to be approved by the FDA and are commercially feasible.

Many chemical phenomena have been proposed as the driving force for CD adduct formation [4–6] with various guest molecules. Most of these mechanisms are related to the effect of the solvent, usually water, on the complex [4]. Clearly, the utility of β-CD in food processing would be affected by the product's water activity. Therefore, to better



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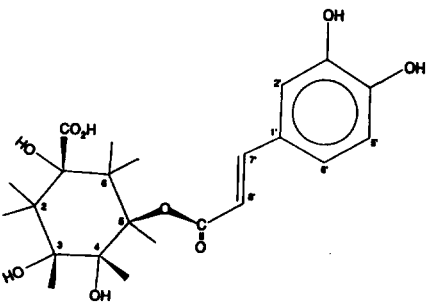

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understand the complexation reaction of **1** with **2** from the standpoint of the solvent, we have performed an investigation of the "solvophobic" [4] effect by studying the dependence of K on water activity, a_{H_2O} (assuming $a_{H_2O} \approx$ the mole fraction of water, X_{H_2O}), using various diluents such as methanol or formamide. We have also utilized ^{1}H inversion recovery NMR spectroscopy in an attempt to determine the localization of that hypothetical [7] species of water which is believed to be uniquely associated with, and which may contribute to, the stability [8,9] of various CD adducts.

2. Experimental

General.—β-CD was donated by the American Maize-Products Company (1100 Indianapolis Blvd., Hammond, IN 46320-1094, USA). Chlorogenic acid (CA) {3-*O*-(3,4-dihydroxycinnamoyl)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, RN = [327-97-9]} was purchased from Sigma Chemical Company. The hydroxyethyl-substituted derivative of **1** (HE-β-CD; degree of substitution ~ 50% as determined by ^{13}C NMR; 53% as reported by Aldrich) was purchased from the Aldrich Chemical Company. Unless otherwise noted, all experiments were performed using 0.1 M Na phosphate buffer (pH ~ 6.7).

^{1}H NMR experiments.—All studies were performed on a Jeol NMR spectrometer operated at 400 MHz ($B_0 = 9.4$ T) in 99.9 atom-% D_2O (MSD Isotopes; 0.1 M Na phosphate buffer). Typical spectrometer conditions were: 16384 data points; 4 kHz spectral width; 4.5 s recycle time. The 90° pulse was measured, and the 5-mm probe's performance was checked whenever the ionic strength (IS) of the solutions was modified. As previously discussed [1], the ^{1}H NMR resonance assignments of **2** were based upon a homonuclear COSY experiment and agree, qualitatively, with those reported [10] for a similar structure. ^{1}H inversion recovery (T_1) peak intensity data (I_i) were fit to a 3-parameter exponential equation,

$$I_i = I_{\infty,i}[1 - 2e^{-(\tau_i - \tau_0)/T_1}],$$

utilizing a modified Gauss–Newton method [11,12] on a Microsoft Excel spreadsheet created in this laboratory [13]; 10 τ values, the time between the 180 and 90° pulses, of 25 to 4800 ms were used. The concentrations of **1** and **2** are provided in the appropriate figure legends or tables. All NMR experiments were performed at ca. 25°C.

UV Experiments.—UV studies were performed on a Shimadzu UV160U spectrophotometer equipped with a CPS cell temperature controller. Thermodynamic experiments utilized a Neslab Endocal refrigerated circulating water bath to maintain the temperature of the various stock solutions ±2°C. Solutions were maintained at a particular temperature for at least 1 h to insure attainment of thermal equilibrium. For measurement of Ks, CA's concentration was fixed at 8×10^{-5} M; β-CD concentrations were 0 (control), 0.125, 0.25, 0.5, 1, 2, 4, 8, 10, and 12 mM in 0.1 M Na phosphate buffer (pH ~ 6.7); HE-β-CD concentrations were 0 (control), 0.25, 0.5, 1, 2, 4, 8, 12, 16, 20, and 24 mM. Samples were prepared by mixing 10 μL of **2** (8 mM) with 0–1000 μL of CD (12 or 24 mM) in a quartz cuvette maintained at the appropriate temperature; the balance of the total volume (1 mL) was made up with buffer alone. Data were acquired shortly after

mixing (ca. 2–5 min.) since CD complex kinetics are typically fast [14] and an apparent chemical equilibrium is reached almost instantaneously. The normal absorption spectrum of **2** was collected and digitally transformed to the 1st derivative whereupon the intercept at $dA(\lambda)/d\lambda = 0$ was recorded (λ_i). $\Delta\lambda_i$, the parameter of interest at each ith CD concentration, was then calculated as

$$\Delta\lambda_i = \lambda_i - \lambda_{\text{Control}}.$$

Calculation of K, ΔH, *and* ΔS.—Since **2** binds to **1** with a 1:1 stoichiometry [1]

$$K = \frac{[\text{CD} \cdot \text{CA}]}{[\text{CD}][\text{CA}]}.$$

Expressing [CD] and [CA] in terms of initial concentrations, $[\text{CD}]_0$ and $[\text{CA}]_0$,

$$K = \frac{[\text{CD} \cdot \text{CA}]}{\{[\text{CD}]_0 - [\text{CD} \cdot \text{CA}]\}\{[\text{CA}]_0 - [\text{CD} \cdot \text{CA}]\}}.$$

If one observes a change in $\Delta\lambda$ as a function of varying $[\text{CD}]_0$,

$$K = \frac{[\text{CA}]_0 \dfrac{\Delta\lambda}{\Delta\lambda_{\max}}}{\left\{[\text{CD}]_0 - [\text{CA}]_0 \dfrac{\Delta\lambda}{\Delta\lambda_{\max}}\right\}\left\{[\text{CA}]_0 - [\text{CA}]_0 \dfrac{\Delta\lambda}{\Delta\lambda_{\max}}\right\}}.$$

Solving for $\Delta\lambda$ provides

$$\left\{\Delta\lambda_{\max}\left(1 + [\text{CD}]_0K + [\text{CA}]_0K - \left[1 + 2[\text{CD}]_0K + 2[\text{CA}]_0K + [\text{CD}]_0^2K^2 + [\text{CA}]_0^2K^2 - 2[\text{CD}]_0[\text{CA}]_0K^2\right]^{1/2}\right)\right\}\{2[\text{CA}]_0K\}^{-1}.$$

The parameters, $\Delta\lambda_{\max}$ and K, were evaluated by fitting the experimental values of $\Delta\lambda$ to the above equation utilizing the modified Gauss–Newton method [11,13]. A plot (Fig. 1) of the average of 3 $\Delta\lambda$ ($\pm$the standard deviation of the mean, $s_{\bar{x}}$) measurements as a function of $[\text{CD}]_0$ demonstrates not only the precision of the technique but also the dependency of $\Delta\lambda_{\max}$ on CD binding site geometry. $\Delta\lambda_{\max}$ may be related to changes in the solvation of **2**'s chromophore upon inclusion [15] and will be discussed herein.

Standard enthalpy and entropy changes due to complexation (ΔH and ΔS, respectively) can be obtained from the dependence of K on temperature (T, K) by way of the Gibbs–Helmholtz equation [16], as shown previously [1], or calculated using nonlinear regression analysis [13]. Using the latter technique, we obtained ΔH and ΔS by fitting observed K values to an exponential equation derived from the well-known relationship,

$$\Delta G = -RT \log_e K = \Delta H - T\Delta S;$$

rearranging and solving for K gives

$$K = e^{-\Delta H/RT}\, e^{\Delta S/R}.$$

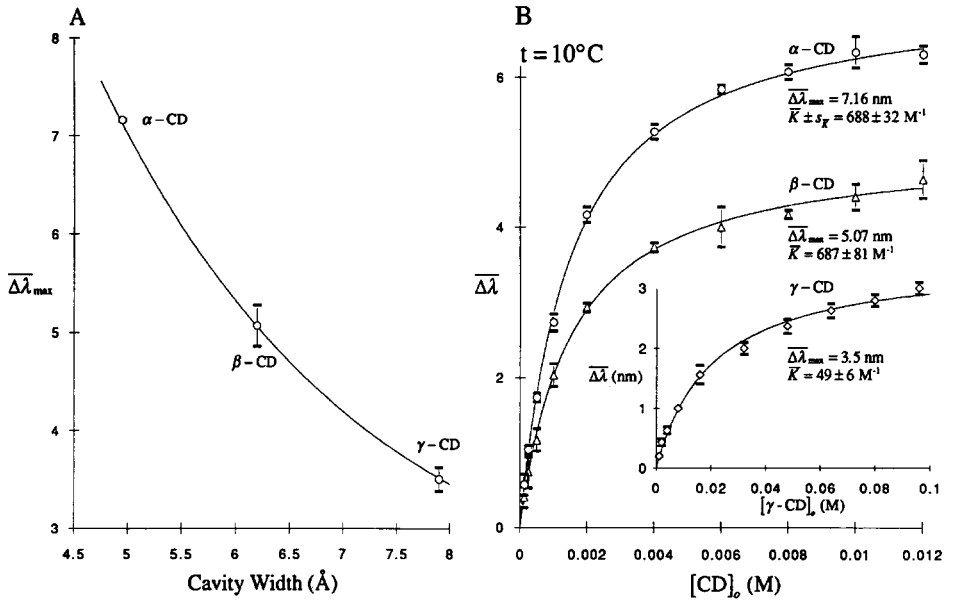

Fig. 1. (A) Plot of calculated average $\Delta\lambda_{max}$ ($\overline{\Delta\lambda}_{max} \pm s_{\bar{x}}$, nm) as a function of CD cavity diameter. (B) Dependency of CA's average $\Delta\lambda$ ($\overline{\Delta\lambda} \pm s_{\bar{x}}$, nm) on $[CD]_0$ (M) at 10°C in 0.1 M sodium phosphate buffer.

Results from either method are similar, but a more meaningful error term, the asymptotic standard error (ε), is obtained by nonlinear regression analysis [13]. These different methods are compared in Fig. 2 (data were computer generated with random error). The dashed curve represents a fit to the above exponential equation using values obtained from the Gibbs–Helmholtz linear regression method ($\Delta H = -22.72 \pm 2.76$ kJ mol^{-1}; $\Delta S = -24.05 \pm 13.53$ J mol^{-1} K^{-1}) whereupon Ks were transformed and then plotted as $\Delta G/T$ versus $1/T$ (Fig. 2 inset). The solid curve represents the best nonlinear fit utilizing the Gauss–Newton method ($\Delta H = -19.78 \pm 1.26$ kJ mol^{-1}; $\Delta S = -10.18 \pm 6.13$ J mol^{-1} K^{-1}). Notice the differences in the way each method, or model, weights the data points. The nonlinear regression technique minimizes the sum of the squares of the differences between the data and the converged solution and therefore weights each data point equally. When plotted on the nonlinear scale, it becomes apparent that the linear regression method weights the data points unequally because of the requisite mathematical transformations to induce linearity.

3. Results and discussion

Inclusion complex formation as a function of X_{H_2O}, *diluent, and hydroxyl group substitution.*—Water activity has substantial effects on CD binding of azo-dyes [5], *p*-nitrophenol [17], and (+)-limonene [18,19]. Related to these observations, we found that there was an isotopic effect on K by substituting D_2O for H_2O [1]. Assuming the

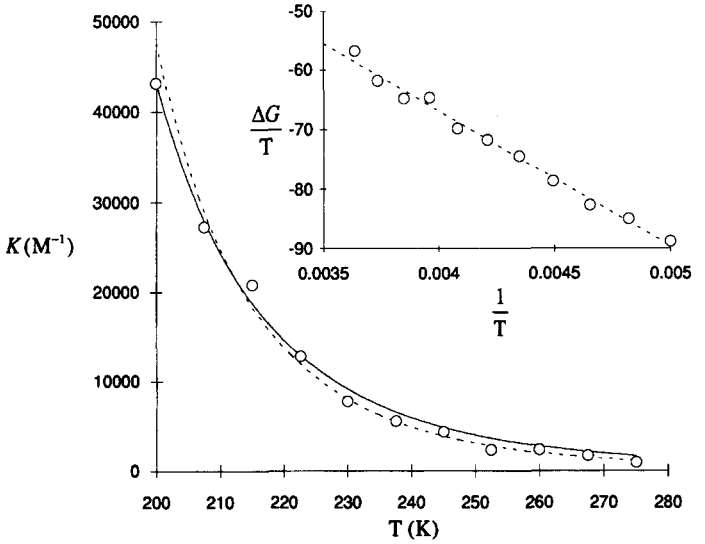

Fig. 2. A comparison of curve fitting K with respect to temperature (T, K) using two computational methods for calculating ΔH and ΔS.

major effect of increasing the activity of any nonaqueous solvent is to decrease a_{H_2O}, all these results [1,5,17] support the equilibrium relationship,

$$CD \cdot [H_2O]_x + Guest \cdot [H_2O]_y + zH_2O \leftrightarrow CD \cdot Guest \cdot [H_2O]_{x'+y'+z},$$

where K is defined as

$$K = K^{\ddagger} a_{H_2O}^{z};$$

in this relationship K, the apparent stability or association constant, is equivalent to the stability constant $K^{\ddagger}$,

$$K^{\ddagger} = \lim_{a_{H_2O} \to 1} K,$$

multiplied by a_{H_2O} to the power of H_2O's stoichiometric coefficient, z. In the above relationship, we have defined the total amount of complex-bound H_2O as $[H_2O]_{x'+y'+z}$ rather than $[H_2O]_{x+y+z}$ since the guest and host probably lose water (e.g., $x > x'$; $y > y'$) upon adduct formation. The small solvent isotope effect on K argues for this model since D_2O H-bonds more strongly than H_2O. The dependency of K on ionic strength [1] (IS) also infers this model since an increase in IS would necessarily perturb the $CD \cdot Guest \cdot [H_2O]_{x'+y'+z}$ H-bond network. Using Me_2SO to modify a_{H_2O}, Gerasimowicz and Wojcik [5] have calculated that z,

$$z = \frac{\partial \log_e K}{\partial \log_e [H_2O]},$$

was ca. 4–9 for several azo dyes (with $[H_2O]$, based here on volume-% not X_{H_2O}). However, these estimates of z might change if the $[H_2O]$ term were converted to the

more physically reasonable X_{H_2O} term. Mole fraction is a more reasonable approximation of a_{H_2O} because all solvents [20] obey Raoult's law as the solute (or diluent) approaches zero concentration. Divergence from this ideal behavior is lessened as the solute approaches the size and chemical characteristics of the solvent [21]. Thus, for a

Table 1
Apparent stability constants (K), maximum bathochromic shifts in absorbance ($\Delta\lambda_{max}$), standard enthalpy (ΔH), and entropy (ΔS) change of CD·CA complexation as a function of temperature (t), mole fraction of water (X_{H_2O}), and CD type

$X_{H_2O}^{MeOH}$ [a]	t (°C)	β-CD			Hydroxyethyl-β-CD		
		$\Delta\lambda_{max}$ (nm) [b]	K (M^{-1})	Thermodynamic parameters [c]	$\Delta\lambda_{max}$ (nm)	K (M^{-1})	Thermodynamic parameters
0.80	5	3.89 ± 0.32	288 ± 66	ΔH:	4.10 ± 0.07	283 ± 20	ΔH:
	10	3.36 ± 0.35	250 ± 67	−11.19 ± 1.88	4.49 ± 0.05	277 ± 11	−16.45 ± 2.12
	15	3.71 ± 0.24	161 ± 22	$\Delta H^{\ddagger}$:	3.85 ± 0.09	215 ± 19	$\Delta H^{\ddagger}$:
	20	3.80 ± 0.22	112 ± 12	−27.31 ± 3.91	3.56 ± 0.09	185 ± 17	−29.29 ± 2.51
	25			ΔS:	3.16 ± 0.10	157 ± 17	ΔS:
				−13.72 ± 6.60			−4.68 ± 7.42
0.84–0.85	5	5.31 ± 0.15	309 ± 25	$\Delta S^{\ddagger}$:	5.10 ± 0.06	425 ± 21	$\Delta S^{\ddagger}$:
	10	4.96 ± 0.09	268 ± 13	−40.31 ± 13.80	5.01 ± 0.08	350 ± 22	−49.72 ± 8.83
	15	4.55 ± 0.06	223 ± 7	T_c:	5.02 ± 0.10	270 ± 18	T_c:
	20	4.20 ± 0.14	219 ± 18	269.12	4.84 ± 0.19	208 ± 26	266.71
	25			α:	4.33 ± 0.17	158 ± 18	α:
				1.11			1.12
0.88–0.90	5	5.47 ± 0.11	455 ± 29	$T\Delta S_0$:	6.32 ± 0.11	546 ± 34	$T\Delta S_0$:
	10	4.93 ± 0.12	433 ± 36	16.52	6.06 ± 0.13	440 ± 31	17.20
	15	4.80 ± 0.11	359 ± 25		5.91 ± 0.13	354 ± 23	
	20	4.89 ± 0.14	223 ± 16		5.86 ± 0.10	274 ± 12	
	25				6.21 ± 0.20	181 ± 13	
0.92–0.95	5	5.48 ± 0.09	636 ± 38		6.44 ± 0.09	668 ± 38	
	10	5.37 ± 0.05	519 ± 17		6.24 ± 0.11	585 ± 38	
	15	5.09 ± 0.09	431 ± 25		6.32 ± 0.17	418 ± 36	
	20	4.99 ± 0.10	330 ± 19		6.35 ± 0.08	378 ± 16	
	25				6.48 ± 0.13	263 ± 14	
0.96–0.99	5	5.52 ± 0.09	793 ± 47		6.63 ± 0.11	753 ± 52	
	10	5.25 ± 0.06	703 ± 35		6.36 ± 0.07	594 ± 24	
	15	5.18 ± 0.06	555 ± 22		6.37 ± 0.20	438 ± 45	
	20	5.20 ± 0.07	412 ± 19		6.13 ± 0.06	390 ± 12	
	25				6.38 ± 0.12	375 ± 21	

[a] $X_{H_2O}^{MeOH}$ values represent the mole fraction of water utilizing MeOH as a diluent (0.1 M sodium phosphate). In these studies we assume $X_{H_2O} \sim a_{H_2O}$.
[b] $\Delta\lambda_{max}$ and K are reported ± the asymptotic standard error (ε).
[c] ΔH and ΔS represent standard enthalpy and entropy changes (± ε) when $X_{H_2O} \sim 1$ (no MeOH). $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were derived (± ε) from the relationship $K^{\ddagger} = e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R}$ whereupon $K^{\ddagger}$ was calculated from the dependency of K on X_{H_2O} ($K = K^{\ddagger} X_{H_2O}^{z}$). All enthalpy and entropy changes are reported in units of kJ mol^{-1} and J mol^{-1} K^{-1}, respectively. $\partial\Delta H/\partial\Delta S$ (T_c; K), $\partial T\Delta S/\partial\Delta H$ (α; unitless), and $T\Delta S_0$ ($\lim_{\Delta H \to 0} T\Delta S$; kJ mol^{-1}) were calculated from all $X_{H_2O}^{MeOH}$ thermochemical measurements.

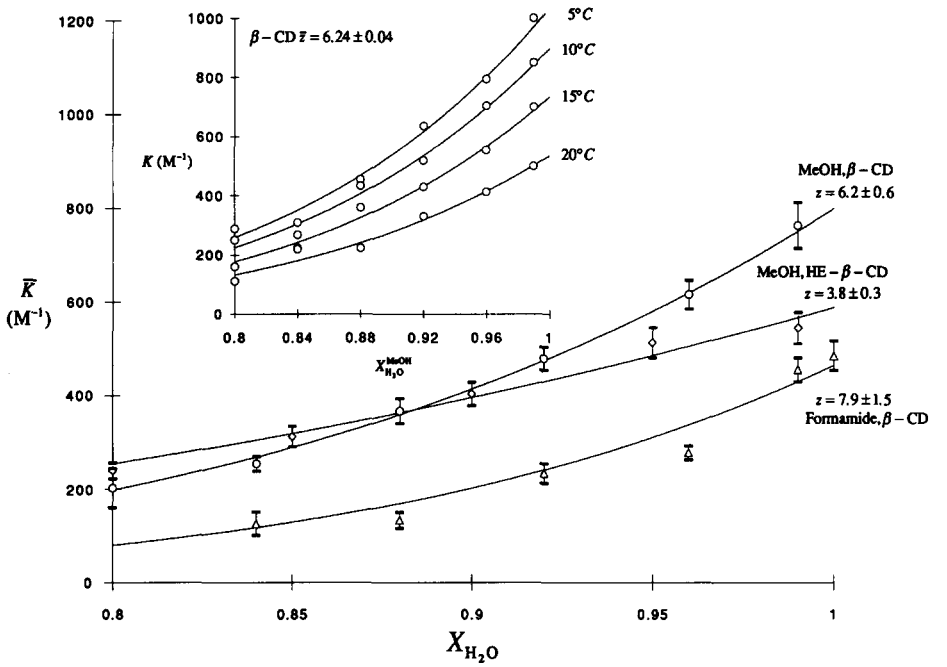

Fig. 3. Relationship ($\bar{K} = \bar{K}^{\ddagger} X_{H_2O}^{z}$) of K averaged across temperature ($\bar{K}$) as a function of X_{H_2O}. For the MeOH/β-CD series $\bar{K}$s were averaged across the t range of 5–20°C (ave. 12.5 ± 6.5°C); for the MeOH/HE-β-CD series the t range was 5–25°C (ave. 15 ± 7.9°C); for the formamide/β-CD series the t range was 10–40°C (ave. 25 ± 13°C); z values are provided ± ε. Higher temperatures were used for the formamide treatments due to limitations in **1**'s solubility under these conditions. Values of z are provided ± ε. Inset: β-CD isothermal changes in K with respect to $X_{H_2O}^{MeOH}$; the average z is provided ± $s_{\bar{z}}$.

chloroform–acetone binary mixture [20], the solvent activity coefficient (γ_a) is close to 1 ($\gamma_a = 0.94$) even at a $X_{solvent}$ as low as 0.8 (e.g., $a_{solvent} \sim X_{solvent}$). For our purposes we have chosen MeOH and formamide as simple diluents to alter X_{H_2O} to no less than 0.8. Using entirely different methods Yoshi et al. [19] have shown that ca. 7 water molecules are needed to stabilize the (+)-limonene · β-CD complex.

Table 1 and Fig. 3 present data on the dependence of β-CD · CA and HE-β-CD · CA complex Ks on X_{H_2O} at various temperatures (t, °C). The Ks for **1** change more (64–73%) with X_{H_2O} than for HE-β-CD (51–62%). The difference in the rate of change in K with respect to X_{H_2O} is more apparent (Fig. 3) when the average K (across t; $\bar{K}$) is plotted as a function of X_{H_2O} for **1** using either MeOH or formamide as diluents or for HE-β-CD using MeOH alone. Using a nonlinear regression technique [21], we found that the average value of z for **1** was about the same using either formamide or MeOH as diluents (7.9 ± 1.5 or 6.2 ± 0.6, respectively) and is remarkably similar ($z = 7$) to what has recently [19] been reported to be the minimum number of water molecules requisite for (+)-limonene · β-CD stability. For HE-β-CD we obtained a z value (3.8 ± 0.3) about half of that observed for **1** in either diluent. This result might indicate that z was associated with H_2O binding to one or more of the CD hydroxyl groups

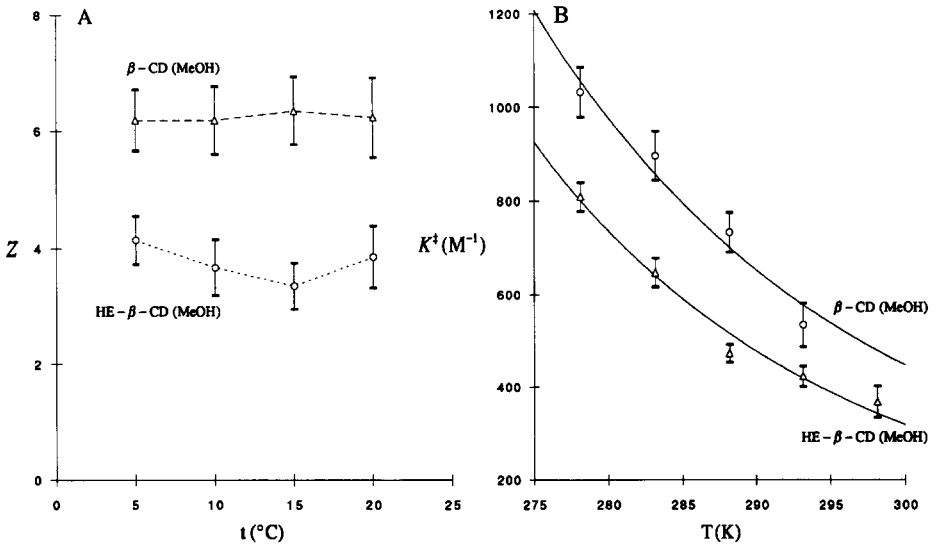

Fig. 4. (A) Effect of temperature (t, °C) on the level of β-CD·CA complex z values over and above the solvation of CA and β-CD alone ($z \pm \varepsilon$). (B) Plot and curve fits of $K^{\ddagger} \pm \varepsilon$ as a function of temperature (T, K) and diluent type.

since a 50% substitution resulted in an equivalent change in z. It should be pointed out that the distribution of hydroxyethyl substitutions may be positionally unequivalent since hydroxypropylation [22] at β-CD ring positions 2, 3, and 6 was found to exist in a molar ratio of ca. 2:1:1.

Using analysis of variance (randomized complete block design [23], blocking on t), we found that there was a statistically significant difference ($F = 158$) in z as a function of CD type but little apparent effect with respect to t (Fig. 4A). Although **1** binds **2** more strongly (Fig. 3 and Fig. 4B) than HE-β-CD, there was not much difference in $\Delta H^{\ddagger}$, derived from the dependence of $K^{\ddagger}$ on T (Table 1), and the $K^{\ddagger}$–T curve fits (Fig. 4B) were therefore nearly parallel.

The average (across t) UV curve fit parameter, $\overline{\Delta\lambda}_{max}$ (Fig. 5), which should be related to a perturbation in the solvation of **2**'s chromophore upon binding and/or a direct interaction with the host's binding site [14,15,24,25], was significantly larger for HE-β-CD (ca. 1 nm) than β-CD in the $X_{H_2O}^{MeOH}$ range of 0.9–0.99. Clearly, $\overline{\Delta\lambda}_{max}$ is related to the CD cavity environment since changes in binding site geometry caused this parameter (Fig. 1) to diminish exponentially as cavity diameter increased from ca. 5 to 8 Å. The change in $\overline{\Delta\lambda}_{max}$ as $X_{H_2O}^{MeOH}$ increased might argue that the CD binding site undergoes a relative desolvation upon adduct formation with **2**. This hypothesis is supported by thermochemical studies to be discussed herein. If the above is true, these results indicate that HE-β-CD surrenders more cavity-bound H_2O, upon complexation, than **1** as $X_{H_2O}^{MeOH} \rightarrow 1$.

Spin-lattice relaxation of the β-CD · CA · $[D_2O]_{x'+y'+z}$ complex.—The spin-lattice relaxation of ^{1}Hs in the extreme narrowing limit is dominated by inter- and intra-molec-

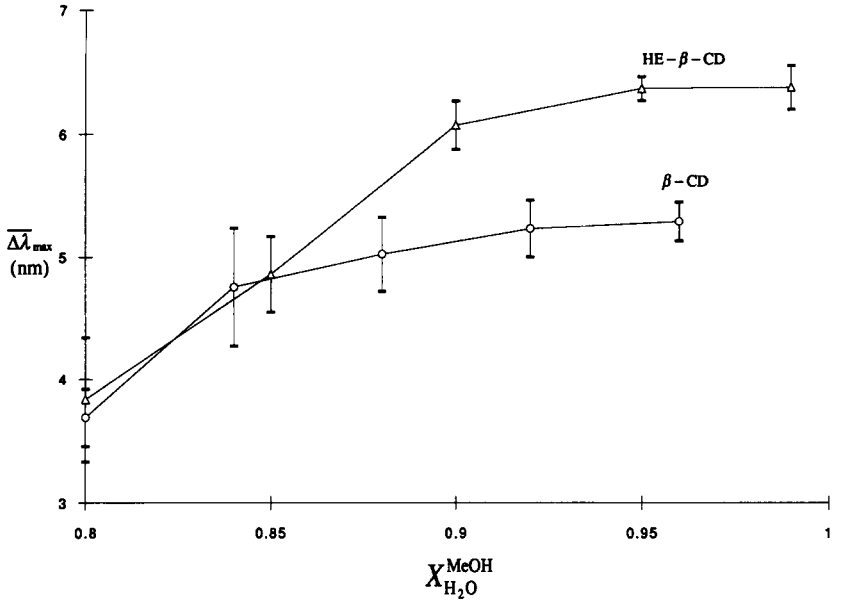

Fig. 5. Dependency of CA's $\overline{\Delta\lambda}_{max} \pm s_{\bar{x}}$ (averaged across t, nm) on the mole fraction of water using MeOH as a diluent ($X_{H_2O}^{MeOH}$) in 0.1 M sodium phosphate buffer. $X_{H_2O}^{MeOH} = [H_2O]/([H_2O]+[MeOH])$.

ular dipolar relaxation. Intramolecular ^1H–^1H relaxation, which is controlled by a molecular motion term (τ_c) and a ^1H–^1H distance term (r_{SS}) [24],

$$T_1 = \frac{2r_{ss}^6}{3\gamma^4\{h/2\pi\}^2\tau_c},$$

has been shown to be negligible for **1** but quite large for certain guests [25]. In our studies, we assume that intramolecular relaxation is an insignificant contributor to **1**'s overall ^1H spin-lattice relaxation processes since only certain resonances (mainly H-3), show substantial changes in T_1 (ΔT_1) upon adduct formation with **2**,

$$\Delta T_1 = T_{1,control} - T_{1,treatment}.$$

The above assumption seems valid since, as a consequence of β-CD's toroidal structure, $^4C_1$ conformation, and lack of free rotation about the glycosidic bonds, any change in intramolecular r_{ss}, or τ_c, due to complexation should affect resonances other than just H-3, especially H-1 and H-4 which, in our studies, are virtually unaltered.

Intermolecular dipole–dipole relaxation is described by

$$T_1 = \frac{27D_{trans}r_{IS}}{16\pi c_S\gamma_I^2\gamma_S^2\{h/2\pi\}^2S(S+1)}$$

or

$$\frac{9D_{trans}r_{SS}}{8\pi c_S\gamma_S^4\{h/2\pi\}^2S(S+1)}$$

for ^{1}H–D- or ^{1}H–^{1}H-induced relaxation, respectively. In the above equations [26]: c_S is the concentration of spin S per unit volume; D_{trans}, which is equal to

$$\tfrac{1}{2}\left(D^{A}_{trans} + D^{B}_{trans}\right),$$

is the mutual translational self-diffusion coefficient of molecule A and B; r is the closest approach of the spins S on molecules A and B. Thus, assuming this form of relaxation is predominant for any β-CD ^{1}H resonance of interest, a significant change in T_1 (e.g., $\Delta T_1 \gg \varepsilon_{T_1}$ or $s_{\bar{x},T_1}$) due to adduct formation would necessarily result from r and D_{trans} diminishing in the treatment with respect to the control,

$$\Delta T_1 \propto \Delta D_{trans}\, \Delta r.$$

Such conditions would occur if there was an increase in the number (e.g., z) of D_2O molecules H-bound to the primary and/or secondary hydroxyl groups of the β-CD · CA adduct. For the equivalent spin model to predominate, CA's H-2′, -5′, or 6′ would have to be positionally close (ca. 2–5 Å) to **1**'s H-3 and H-5. Of course, the mean lifetime of these intermolecular interactions would also have to be long on the NMR timescale (e.g., ≥ ca. 2.5 ns at 400 MHz) [27].

If changes in β-CD's T_1 result from intermolecular ^{1}H–D relaxation, then ΔT_1 is a direct measure of the localization of complex-bound D_2O and, therefore, z. However, as mentioned previously, relaxation is not mechanistically trivial and there may be some, possibly significant, contribution to ΔT_1 from ^{1}H–^{1}H intermolecular relaxation (e.g., **1** ↔ **2**) whereupon the H-3 and H-5 of **1**, which form rings along the outer edges of the CD annulus [22], would be a likely species to interact with the aromatic spins of **2**. If D_2O were bound with a long life-time of association with the β-CD · CA complex, one would expect only small ΔT_1s to occur for any resonance other than the H-2, -3, and/or -6,6′ of **1**. We have found that the largest ΔT_1 (Table 2) was associated with the H-3 of **1** (585 ± 74 ms). Smaller ΔT_1s were also observed for H-2 (160 ± 50 ms) and H-6,6′ (83 ± 12 ms). The relatively large H-2 ΔT_1 argues against a ^{1}H–^{1}H intermolecular relaxation mechanism, exclusively, since these ^{1}Hs are outward facing and, therefore, a

Table 2
T_1s and ΔT_1s for the β-CD·CA complex as a function of varying degrees of binding site occupation ($X_{\beta\text{-CD}} = 0.2$ and 0.8).

Resonance	T_1 (ms)			ΔT_1 (ms)	
	Control [a]	$X_{\beta\text{-CD}} = 0.2$ [b]	$X_{\beta\text{-CD}} = 0.8$ [c]	$X_{\beta\text{-CD}} = 0.2$	$X_{\beta\text{-CD}} = 0.8$
β-CD H-1	748 ± 29	731 ± 38	740 ± 7	17	8
β-CD H-3	1381 ± 103	796 ± 56	1005 ± 64	585	376
β-CD H-6,6′	459 ± 12	376 ± 15	412 ± 8	83	47
β-CD H-2	1223 ± 50	1063 ± 81	1147 ± 19	160	76
β-CD H-4	729 ± 24	771 ± 44	709 ± 8	−42	20

[a] Averaged across concentration ± $s_{\bar{x}}$. There was no apparent effect of concentration on T_1.
[b] $T_1 \pm \varepsilon$. [β-CD]$_0$ + [CA]$_0$ = 12 mM; [β-CD]$_0$ = 0.2 × 12 mM (ca. 20% guest-bound and 80% CD sites occupied; 0.1 M sodium phosphate, 25°C, $K_{D_2O} = 605 \pm 52\ M^{-1}$).
[c] [β-CD]$_0$ + [CA]$_0$ = 12 mM; [β-CD]$_0$ = 0.8 × 12 mM (ca. 80% guest-bound and 20% CD sites occupied).

Table 3
^{1}H T_1s and ΔT_1s of **1** when $X_{\beta\text{-CD}} \sim 0.02$ and IS = 0.22 M

Resonance [a]	T_1 (ms)		ΔT_1 (ms)
	β-CD [b]	β-CD + CA [c]	
β-CD H-1	751 ± 25	837 ± 24	−87
β-CD H-3	1439 ± 51	1158 ± 30	281
β-CD H-5	732 ± 105	717 ± 9	15
β-CD H-2	1246 ± 77	1252 ± 47	−7
β-CD H-4	747 ± 24	774 ± 11	−27

[a] β-CD resonance H-6,6′ was obscured by CA's H-4.
[b] All observations are the mean of 4 replicates ± $s_{\bar{x}}$.
[c] $[\beta\text{-CD}]_0 = 2.4 \times 10^{-3}$ M; $[\text{CA}]_0 = 9.6 \times 10^{-2}$ M.

$\text{CA}_{\text{H-2',5',6'}}$–$\beta\text{-CD}_{\text{H-2}}$ distance parameter (r_{SS}) would necessarily be large. ΔT_1s were also observed to diminish as $X_{\beta\text{-CD}}$,

$$X_{\beta\text{-CD}} = \frac{[\beta - \text{CD}]_0}{[\beta - \text{CD}]_0 + [\text{CA}]_0},$$

increased and was presumably due to an enlargement in the population of **1**s with unoccupied binding sites. Neither the H-1 nor H-4 resonances of β-CD displayed any significant perturbation in T_1. Ordinarily the H-5 resonance of **1** is partially obscured by H-6,6′; as the population of β-CD's binding sites fill, H-5 shifts up field to a maximum of ca. 34 Hz and is the largest observed $\Delta\delta$ for β-CD [1]. However, at a high IS (Table 3), we found that the H-5 was resolved from both **1**'s H-6,6′ and **2**'s H-4. If ^{1}H–^{1}H intermolecular relaxation changes were the dominant mechanism for our observed β-CD ΔT_1s (Table 2), one would expect the H-5 of **1** to also show a significant ΔT_1 since changes in chemical shift ($\Delta\delta_{\beta\text{-CD,H-5}}$), due to complex formation, for these resonances were large [1]. However, in Table 3 we see that the ΔT_1 for H-5 under these conditions was insignificant ($\Delta T_1 = 15 \pm 57$ ms), indicating that the ^{1}H–^{1}H intermolecular relaxation mechanism was not predominant. It is interesting and perhaps significant that all of **1**'s resonances except H-3 displayed small (or negative) ΔT_1s at a high IS. IS would affect the β-CD · CA · $[\text{D}_2\text{O}]_{x'+y'+z}$ complex's H-bonding [1] and should result in the equilibrium shifting more toward dissociation (i.e., a smaller K).

If β-CD's H-3 ΔT_1 is associated with rim-bound z D_2Os, then it should diminish as IS is increased. Fig. 6 demonstrates that **1**'s H-3 ΔT_1 diminished ca. 40% as the IS of the solution was increased from 0.06 to 0.22 M. Only insignificant changes were associated with the H-4 resonance (Fig. 6, insert). The inversion recovery intensity data ($\pm s_{\bar{x}}$) and associated curve-fits in Fig. 6 graphically illustrate that even the greatest perturbations in T_1 are relatively small [28] as would be expected for only subtle shifts (e.g., $z \sim 7$) in the overall hydration of the β-CD · CA complex.

Entropy–enthalpy compensation behavior in the methanol–water binary solvent system.—Enthalpy–entropy compensation [7] is a term utilized to describe the behavior of ΔH and ΔS for a series of similar reactions driven by changes in solvation. In particular, the enthalpy change for such processes is linearly correlated with changes in

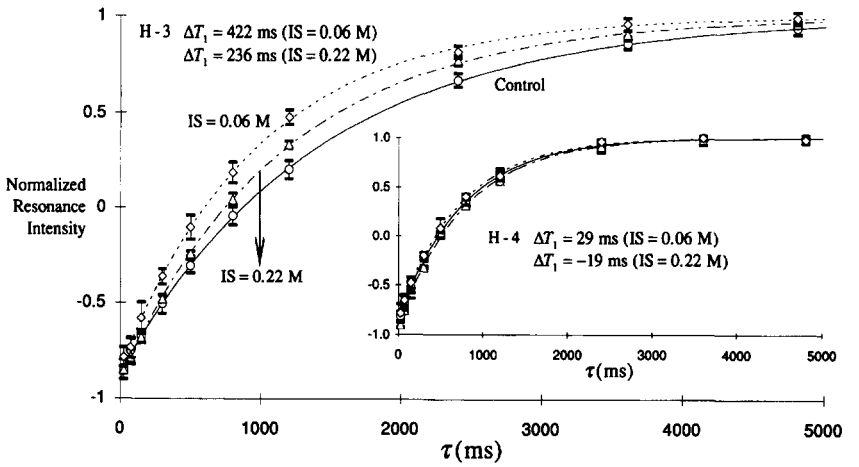

Fig. 6. Effect of IS upon the spin-lattice relaxation time of β-CD's ^{1}H resonances at H-3 and -4 (inset). Control treatments consisted of 4 replicates (mean intensity $\pm s_{\bar{x}}$) each of either 2.4×10^{-3} or 10^{-4} M β-CD at 2 ISs. The low IS ($\frac{1}{2}\Sigma_i M_i c_i^2$; c_i = charge and M_i = molar concentration of each ith constituent) treatments represent the mean of 4 replicates $\pm s_{\bar{x}}$: $[\beta\text{-CD}]_0 = 2.4\times10^{-4}$ M, $[CA]_0 = 9.6\times10^{-3}$ M and sodium phosphate = 0.03 M. The high IS treatments represent the mean of 4 replicates $\pm s_{\bar{x}}$: $[\beta\text{-CD}]_0 = 2.4\times10^{-3}$ M, $[CA]_0 = 9.6\times10^{-2}$ M and sodium phosphate = 0.1 M.

entropy. According to Lumry and Rajender [7], this linear relationship is in no way an outcome of thermodynamic law since, for any ith species in a simple reaction, ΔS_i and ΔH_i are related by the thermodynamic expressions,

$$\Delta H_i = \Delta H_i^o + \int_{T^o}^{T} \Delta C_{p_i}\, \mathrm{d}T$$

and

$$\Delta S_i = \Delta S_i^o + \int_{T^o}^{T} \frac{\Delta C_{p_i}}{T}\mathrm{d}T,$$

whereupon the superscript "o" represents some initial reference state. These relationships are linear with one another only in the "trivial" [7] case where ΔC_p is zero for all i. Thus, the extrathermodynamic [7] compensation behavior described herein and elsewhere [1,7–9,24] is indicated if a simple linear relationship exits between ΔS and ΔH even though the ΔC_ps are non-zero. Typically, a series of ΔH–ΔS pairs for association/dissociation reactions (even in proteins) are created by altering the solvent with monohydroxy alcohols [7], changing the pH [1,7] with different buffers, or by working with different forms of the interacting species [7–9] (e.g., derivatives of CD). However such ΔH–ΔS pairs are created, all of these methods should produce only one linear relationship [7] for each class of chemical interaction.

In processes such as CD · guest adduct formation, where perturbations in the solvation of both guest and host may play a role in determining inclusion complex stability [24], a plot of ΔH versus ΔS (Fig. 7) or $T\Delta S$ versus ΔH [8,9] (Fig. 7 inset) should

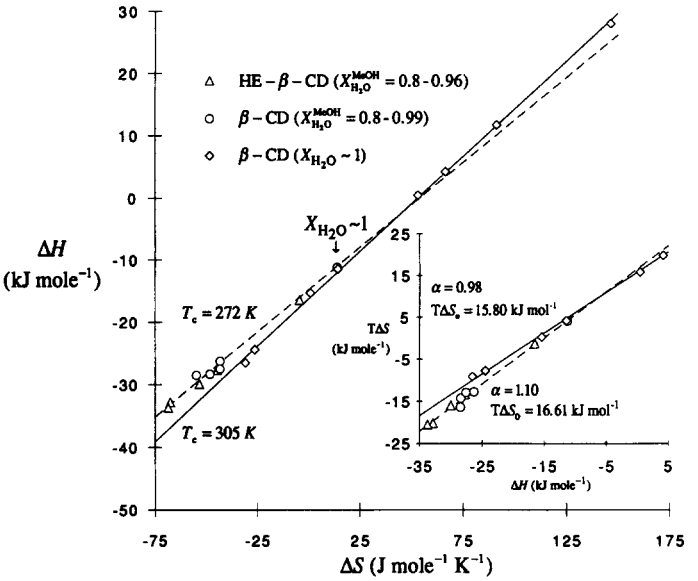

Fig. 7. Enthalpy–entropy compensation plots (ΔH versus ΔS) for CD·CA inclusion complexes using MeOH as a diluent (circles and triangles); Previously published β-CD·CA inclusion complex thermodynamic studies (Ref. 1: diamonds; $X_{H_2O} \sim 1$, buffer only) are provided for comparison. Inset: TΔS (T = 298 K) versus ΔH enthalpy–entropy compensation plot.

result in a straight line. The slope ($\partial\Delta H/\partial\Delta S$) of the former (Fig. 7), known as the compensation temperature (T_c), has been shown to be ca. 300 K [24] for many different CD adducts. Fig. 7 shows calculated values of ΔH plotted against ΔS for various types of β-CD · CA complexes [1] (diamonds) where $X_{H_2O} \sim 1$ (buffer alone) along with data from the variable $X_{H_2O}^{MeOH}$ studies (circles and triangles). The uppermost (dashed) line represents the least-squares fit for the thermodynamic parameters utilizing MeOH to alter X_{H_2O} (T_c = 272 K). The lowermost line in Fig. 7 represents the line of best fit for data [1] on β-CD · CA complexation (T_c = 305 K). A similar thermodynamic parameter [8,9], α, ($\alpha = T\partial\Delta S/\partial\Delta H = 1.1$; T = 298 K; Fig. 7 inset), calculated from changes in $T\Delta S - \Delta H$ pairs measured at various $X_{H_2O}^{MeOH}$s, was greater than α derived from standard aqueous thermodynamic measurements ($\alpha = 0.98$) in a similar system. It is interesting to note that, generally, as one follows the monotonic progression from most to least negative ΔH–ΔS pairs (Fig. 7), the data points positionally progress from low to high $X_{H_2O}^{MeOH}$ values. A similar ΔH–ΔS compensation pair ordering has been noted before [7] for the proton dissociation from simple alkyl ammonium compounds ($T_c \sim 300$ K; $\alpha \sim 0.99$) and results from differences in the interaction of the hydrophobic groups and H_2O. Thermodynamically, any process, no matter how simple, can be considered to be a sum of "part processes" [7]. For our system these parts should consist of a "chemical" part process and a "solvation" part process. According to Lumry and Rajender [7] the mutual nonlinearity ($X_{H_2O} < 1$ data falling on differing lines than $X_{H_2O} \sim 1$ data) in our compensation data may result from more than one part process

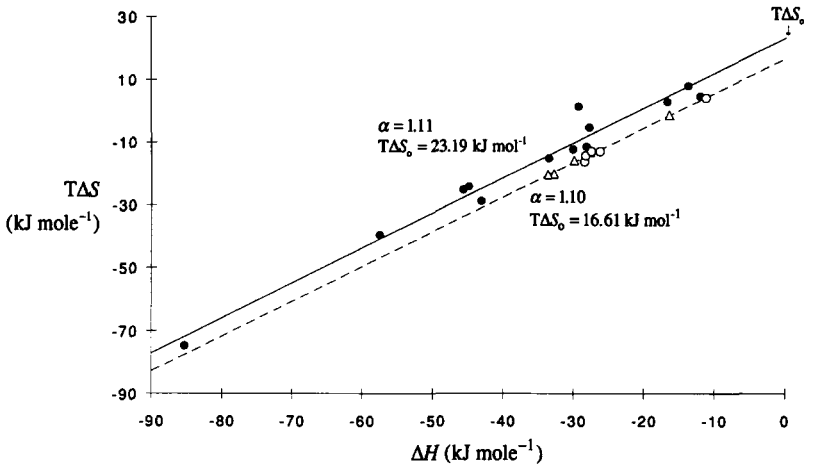

Fig. 8. Enthalpy–entropy compensation plots for CD·CA inclusion complexes using MeOH as a diluent (circles and triangles); data from modified β-CD·2-naphthalene sulfonate inclusion complex studies (Ref. 8: closed circles) are provided for comparison. All calculations were performed for T = 298 K.

depending upon solvent composition. Thus, for β-CD (Fig. 7), the chemical part process may be thermodynamically linked to the partial desolvation of the CD cavity. This hypothesis is supported by the dependency of $\overline{\Delta\lambda}_{max}$ on CD binding site geometry (Fig. 1) and $X_{H_2O}^{MeOH}$ (Fig. 5).

The intercept ($T\Delta S_0$) of the entropy–enthalpy compensation plot (Fig. 7 inset and Fig. 8), which has been interpreted [7–9] as being related to changes in the chemical part process of the CD host–guest interaction, was only somewhat larger ($T\Delta S_0$ = 16.61 kJ mol^{-1}) when $X_{H_2O} < 1$ than when measured at $X_{H_2O} \sim 1$ ($T\Delta S_0$ = 15.8 kJ mol^{-1}) and argues that the MeOH levels we have used do not significantly alter the chemistry of the host–guest interaction. Thus, the differences we observe in T_c when $X_{H_2O} < 1$ appear to be exclusively related to changes in the solvation of the host, guest and complex. Other investigations [8] into the effect of CD modification, in aqueous solutions, have observed an α (Fig. 8, closed circles; $\alpha = 1.11$) almost identical to ours. However, in these same studies Inoue and co-workers [8] detected a larger $T\Delta S_0$ (23.19 kJ mol^{-1}) for this series of variably substituted **1**s. Calculations in Table 1 show that the hydroxyethyl derivative of **1** had only slightly larger values for α and $T\Delta S_0$ (1.12 and 17.2 kJ mol^{-1}, respectively) than β-CD (1.11 and 16.52 kJ mol^{-1}). Both αs and $T\Delta S_0$s, reported herein (α = 1.11, $T\Delta S_0$ = 16.52 kJ mol^{-1}; Table 1) and elsewhere [1] (α = 0.98, $T\Delta S_0$ = 16.61 kJ mol^{-1}; Fig. 7 inset) for unmodified β-CD · CA equilibria, were larger than those recently published [9] (α = 0.9, $T\Delta S_0$ = 12.97 kJ mol^{-1}) for unmodified α-, β-, and γ-CD binding of various napthalenesulfonates.

4. Conclusions

In this manuscript we have presented data which argues that water plays a major structural role in stabilizing the CD · CA adduct. Firstly, from the dependence of K on

X_{H_2O}, we estimated that the β-CD · CA complex's stoichiometric coefficient for water was ca. 7 and argued that this apparent bound water was associated with the CD hydroxyl groups since a 50% substitution resulted in an equivalent loss in z. Confidence in this interpretation results from the fact that other workers [19] have recently shown that an equivalent amount of bound water is requisite for the stability of a similar complex. Secondly, the formation of the adduct alone perturbs β-CDs ^{1}H spin-lattice relaxation time (ΔT_1) mainly at the H-3 position and ΔT_1 is lessened under conditions which have classically been associated with the breaking of H-bond networks and which lessen K. Lastly, thermodynamic data show that T_c ($X_{H_2O} < 1$) was significantly lower (33 K) than an equivalent measure at the limit $X_{H_2O} \rightarrow 1$. This observation is different than that noticed for other systems [7] which involve only simple solvation alone.

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