Thermodynamics of Binding of Guest Molecules to α- and β-Cyclodextrins

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Values of ΔG, ΔH, and ΔS are reported for the interaction of eighteen molecules and ions with α-cyclodextrin and of four molecules with β-cyclodextrin. The conclusions of this study are (1) the cyclodextrin–ClO_4^- complex has been shown to bind a cation, (2) α- and β-cyclodextrin have equilibrium constants for binding the same guest that are similar but the enthalpy and entropy changes are quite different in some cases, and (3) changes in ΔH are largely compensated for by changes in ΔS and it is suggested that this effect is due principally to the nature of the solvent, *i.e.* water.

A SCHEMATIC representation of the structure of α-cyclodextrin is given in Figure 1. The principle difference between α- and β-cyclodextrin is the size of the cavity in the centre of the molecule. The diameter of the ' hole ' in α-cyclodextrin is *ca.* 6 Å while the ' hole ' for β-cyclodextrin has a diameter of *ca.* 7·5 Å.[1]

The ability of the cyclodextrins to form ' inclusion compounds ' or ' clathrates ' in aqueous solution with a variety of inorganic and organic compounds has been investigated by a number of workers.[2-4] The crystalline iodine– and potassium acetate–cyclodextrin complexes have been studied by *X*-ray diffraction and both iodine and potassium acetate have been found to reside within the cavity of α-cyclodextrin in the solid state.[5,6] There is no direct evidence that the cyclodextrin complexes in aqueous solution consist of the guest molecule fixed within the cavity of the cyclodextrin host molecule; however, this picture of the complex is generally accepted. The binding forces between the cyclodextrins and the guest molecule are provided by hydrogen bonding,[7] non-specific van der Waals forces,[8] and hydrophobic interactions.[9]

This phenomenon has stimulated a great deal of interest in the cyclodextrins as models for enzyme

[1] F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Amer. Chem. Soc.*, 1967, **89**, 14.
[2] ' beta-Cyclodextrin,' Corn Products Development, Englewood Cliffs, 1968.
[3] D. French, *Adv. Carbohydrate Chem.*, 1957, **12**, 189.
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[6] A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, 1965, **87**, 2779.
[7] F. Cramer and W. Kampe, *J. Amer. Chem. Soc.*, 1965, **87**, 1115.
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substrate binding.[10-14] Studies of cyclodextrin catalysis of diphenyl pyrophosphate cleavage [10] and phenyl ester cleavage [13,14] have led to the conclusion that these reactions are good models for the action of chymotrypsin.

The object of this investigation is to obtain values for the free energy, enthalpy, and entropy changes for the formation of some cyclodextrin complexes and to discuss these results in terms of the hydrophobicity of the guest molecules and preferential solvation.[15]

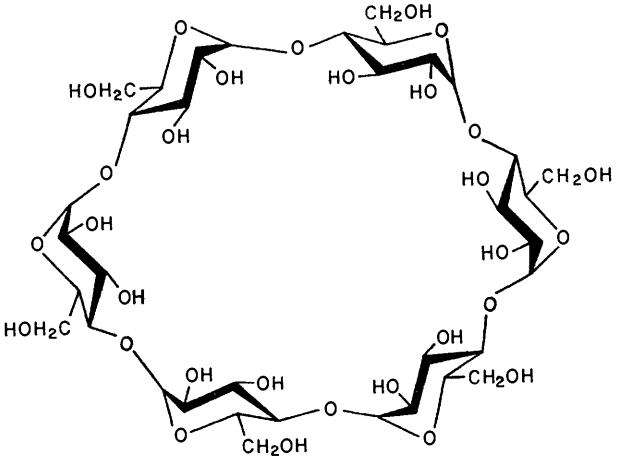

FIGURE 1 Schematic representation of the structure of α-cyclodextrin. α- and β-cyclodextrin are cyclic polymers containing respectively six and seven anhydroglucose units joined by α-1,4-glucosidic linkages. The physical shape approximates to a torus with a central cavity of *ca.* 6 for α- and 7—8 Å diameter for β-cyclodextrin

EXPERIMENTAL

The measurements were made using a Tronac thermometric titration calorimeter equipped with a reaction vessel (5 ml) and modified as described in ref. 16. The Wheatstone bridge was operated at 12 V and the resulting sensitivity was 139·5 mV K^{-1} which was constant over a 1 K range. All experiments were done with the burette and reaction vessel in a constant-temperature bath maintained at 298·1 ± 0·1 K and controlled to ±0·0005 K. A typical run consisted of 20 data points taken in the fore period, 50 data points taken in the main period and 20 data points taken in the after period all at intervals of 10 s. The burette delivery rate selected for this study was 0·1107 ml min^{-1}.

As determined by chemical calibration for the reaction of NaOH (*ca.* 0·01M) and TRIS (trishydroxymethylaminomethane) (*ca.* 0·01M) with $HClO_4$ (*ca.* 0·05M), the reaction vessel had an energy equivalent, ε, of 0·0295 ± 0·0003 cal mV^{-1} when containing 3·99 ml of the TRIS or NaOH solution. An iterative calculation procedure was used to find the value of ε which would produce the correct values for the enthalpy of ionization of water (13·33 kcal mol^{-1}) [16] and the enthalpy of protonation of TRIS (−11·32 kcal mol^{-1}).[16] This energy equivalent (0·0295 cal mV^{-1}) was assumed to be valid for the vessel when it contained 3·99 ml of the other dilute aqueous solutions used in this study. Electrical calibration gave a slightly higher value of ε, 0·0301 ± 0·0002 cal mV^{-1}, apparently because of heat loss in the heater leads.

It was determined by titration of the cyclodextrins into water and by titration of the various guest molecules with water that all heat of dilution corrections were negligible.

All the experiments were carried out as follows. The reaction vessel was charged with 3·99 ml of a *ca.* 0·05M solution of the guest molecule. After an equilibration time of *ca.* 5 min the fore part of the run was started. In the main period 0·9232 ml of either 0·0489M-α-cyclodextrin or 0·0142M-β-cyclodextrin titrant was added to the reaction vessel.

A complete description of the equipment and procedures and a complete listing of all data obtained are available.[17]

Calculations.—The general methods used to reduce the temperature–time calorimetric data and to calculate equilibrium constants and ΔH values simultaneously from enthalpograms have been described.[18-20] Specifically, a grid search method was used to find the minimum in the error square sum functions of the incremental heat changes. The use of increments between data points instead of the total heat at each data point was found to weight the experimental data more evenly than using the total heat at each data point from the beginning of the titration.[20] The error square sum functions for all the experiments reported here had a single minimum of 10—50 μcal^2 for the 50 data points taken.

It was assumed that only one simple reaction (*i.e.* one cyclodextrin molecule and one guest molecule ⟶ complex) was taking place during these experiments. This assumption may not be correct in some cases but was used for lack of a better model. Most previous studies have found that the stoicheiometry of the cyclodextrin complexes is fixed and that the mole ratio of host to guest is 1 : 1 (*e.g.* see ref. 1).

The details of the calculations and listings of the computer programs used (IBM360-67–FORTRAN) are available.[17]

Materials.—All chemicals, with the exception of α- and β-cyclodextrin, used in this study were the best commercial grade available and were used without further purification: phenol (Allied Chemical); benzoic acid (Mallinkrodt); aniline (distilled immediately before use) and pyridine (Fisher Scientific); 2-aminobenzoic acid (Matheson); 4-aminobenzoic acid, 3-methylbenzoic acid, 2-nitrophenol, and 4-nitrophenol (Eastman); L-mandelic acid and hydrocinnamic acid (Aldrich); indole, L-phenylalanine, L-tyrosine, and L-tryptophan (Calibiochem); sodium perchlorate, sodium chloride, acetic acid, and perchloric acid (Baker). All the guest molecule stock solutions were made and used within 24 h.

The cyclodextrins were generously supplied by the Corn

[10] N. Henrich and F. Cramer, *J. Amer. Chem. Soc.*, 1965, **87**, 1121.
[11] M. L. Bender, R. L. Van Etten, G. A. Cowles, and J. F. Sebastion, *J. Amer. Chem. Soc.*, 1966, **88**, 2318.
[12] M. L. Bender, R. L. Van Etten, and G. A. Cowles, *J. Amer. Chem. Soc.*, 1966, **88**, 2319.
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[17] E. A. Lewis, Ph.D. Dissertation, 1972, University of New Mexico.
[18] J. J. Christensen, R. M. Izatt, L. D. Hansen, and J. A. Partridge, *J. Phys. Chem.*, 1966, **70**, 2003.
[19] L. D. Hansen and E. A. Lewis, *Analyt. Chem.*, 1971, **43**, 1393.
[20] D. J. Eatough, J. J. Christensen, and R. M. Izatt, *Thermochimica Acta*, 1972, **3**, 219.

Products Development division of Corn Products Co., Englewood Cliffs, New Jersey. α-Cyclodextrin, as obtained, contained 15% β-cyclodextrin and was purified by the crystallization method of French *et al.*[21a] β-Cyclodextrin was relatively pure, as obtained, but was recrystallized twice from water to remove any γ- and α-cyclodextrin present.

All solutions were prepared using doubly distilled and boiled H_2O. The concentrations of all stock solutions were calculated on the basis of the weight of the solute. The concentrations of the cyclodextrin titrant solutions were checked from the optical rotation of sodium light (α-cyclodextrin, α_D +150·5 ± 0·5°; β-cyclodextrin, α_D +162·5 ± 0·5°).[21a] The observed rotations agreed with the concentrations as determined by weight.

aqueous solutions of approximately zero ionic strength and therefore can be assumed to be equal to ΔG_b^0, ΔH_b^0, and ΔS_b^0 for the particular reactions considered at 298 K.

The error limits given in Tables 1 and 2 are standard deviations of the mean for a series of four runs for the α-cyclodextrin experiments (Table 1) and for a series of three runs for the β-cyclodextrin experiments (Table 2). In some cases the error limits are quite large (*ca.* 2 kcal mol^{-1} for both ΔG and ΔH). These limits are the result of several factors. The accuracy of these experiments was limited by the necessity of using quite dilute solutions because of limited solubility of the cyclodextrins and many or the guest compounds. Also, in some cases the small ΔH values resulted in very small temperature rises (*ca.* 0·01 K) which limited the accuracy of the determinations.

TABLE 1

Thermodynamics of binding of guest molecules to α-cyclodextrin at 298·1 K

Guest molecule	log K (formation)	ΔG_b/kcal mol^{-1}	ΔH_b/kcal mol^{-1}	ΔS_b/cal mol^{-1} K^{-1}
Phenol (1)	4·2 ± 1·3	−5·7 ± 1·8	−1·8 ± 0·2	+13
Benzoic acid (2)	3·0 ± 0·1	−4·1 ± 0·1	−9·6 ± 0·1	−18
Indole (3)	7·8 ± 0·1 [a]	−10·6 ± 0·1	−0·8 ± 0·1	+33
Hydrocinnamic acid (4)	3·1 ± 0·1	−4·2 ± 0·1	−7·5 ± 0·1	−11
L-Phenylalanine (5)	4·1 ± 1·4	−5·6 ± 1·9	−1·1 ± 0·3	+15
L-Tyrosine (6)	2·9 ± 0·6	−4·0 ± 0·8	−1·0 ± 1·0	+10
L-Tryptophan (7)	1·5 ± 0·1	−2·0 ± 0·1	−1·8 ± 0·1	+1
Aniline (8)	*b*			
Anilinium perchlorate (9)	1·5 ± 0·2	−2·0 ± 0·3	−12·3 ± 1·9	−35
Perchloric acid (10)	1·6 ± 0·1	−2·2 ± 0·1	−7·5 ± 1·0	−17
Sodium perchlorate (11)	1·3 ± 0·1	−1·8 ± 0·1	−9·7 ± 1·2	−23
	(1·5) [c]	(−2·0) [c]		
Sodium chloride (12)	*b*			
2-Aminobenzoic acid (13)	5·0 ± 1·3	−6·8 ± 1·8	−0·3 ± 0·1	+21
4-Aminobenzoic (14)	2·8 ± 0·1	−3·8 ± 0·1	−11·6 ± 0·5	−26
3-Methylbenzoic acid (15)	2·4 ± 0·1	−3·3 ± 0·1	−11·6 ± 0·9	−28
4-Nitrophenol (16)	2·1 ± 0·3	−2·9 ± 0·4	−7·3 ± 1·5	−15
	(2·6) [c]	(−3·6) [c]	(−4·2) [c]	(−2) [c]
2-Nitrophenol (17)	3·7 ± 1·1	−5·0 ± 1·5	−0·5 ± 0·1	+15
L-Mandelic acid (18)	2·3 ± 1·4	−3·1 ± 1·9	−4·9 ± 2·5	−6
Acetic acid (19)	3·8 ± 1·2	−5·2 ± 1·6	−1·2 ± 0·1	+13
Pyridine (20)	2·2 ± 1·4	−3·0 ± 1·9	−2·5 ± 0·9	+2

[a] Precision limit given; the accuracy, however, is of the order of ±3 units because of possible systematic errors in the calculations at values of log $K > 5$. [b] Indicates that either no reaction takes place between the guest and α-cyclodextrin or that a negligible temperature change ($< 5 \times 10^{-4}$ K) occurs during the reaction. [c] Data from ref. 1, for 4-nitrophenol are at 14 °C and at 20 °C for $NaClO_4$.

RESULTS

The free energy changes ΔG_b, enthalpy changes ΔH_b, and the entropy changes ΔS_b for the binding of the various guest molecules to either α- or β-cyclodextrin are given in Tables 1 and 2, respectively. These values were determined in dilute

TABLE 2

Thermodynamics of binding of guest molecules to β-cyclodextrin at 298·1 K

Guest molecule	log K (formation)	ΔG_b/ kcal mol^{-1}	ΔH_b/ kcal mol^{-1}	ΔS_b/ cal mol^{-1} K^{-1}
(1)	3·4 ± 1·0	−4·6 ± 1·4	−2·6 ± 0·2	+7
(2)	2·1 ± 0·4	−2·9 ± 0·5	−7·6 ± 2·7	−16
(15)	7·1 ± 0·1 [a]	−9·6 ± 0·1	−11·7 ± 1·2	−7
(16)	3·0 ± 1·7	−4·1 ± 2·3	−10·5 ± 2·5	−21
(4)	*b*			
	(2·3) [c]	(−3·2) [c]		
(3)				
(14)	*b*			
(5)	*b*			

[a] Precision limit given; the accuracy, however, is of the order of ±2 units because of possible systematic errors in the calculations at values of log $K > 5$. [b] Indicates that either no reaction takes place between the guest and β-cyclodextrin or that a negligible temperature change ($< 5 \times 10^{-4}$ K) occurs during the reaction. [c] Data, from ref. 22, are at 30 °C.

The accuracy and precision obtainable in the 5 ml reaction vessel (3 mcal + 1%) is less than that obtainable with 100 or 25 ml vessels (20 mcal + 0·2%). However it was decided to use the 5 ml vessel since the amount of α-cyclodextrin that could easily be purified would have limited the number of guest compounds that could have been studied in a larger vessel, *i.e.* some accuracy was traded for the ability to survey more reactions.

A complete discussion of the limitations and errors involved in the simultaneous calculation of equilibrium constants and ΔH values from calorimetric data has been given previously.[21b]

Another factor affecting the accuracy, but not the precision, is the possibility that reactions other than the 1 : 1 binding of guest molecules to cyclodextrin are taking place. Other possible reactions which have not been taken into account in this study are the sandwiching of the guest molecule between two cyclodextrin molecules or the interaction of two guest molecules with one cyclodextrin

[21] (*a*) D. French, M. L. Levine, J. H. Pazur, and E. Norberg, *J. Amer. Chem. Soc.*, 1949, **71**, 353; (*b*) J. J. Christensen, D. P. Wrathall, J. O. Oscarsen, and R. M. Izatt, *Analyt. Chem.*, 1968, **40**, 1713.

molecule. Also, since many of the guest molecules are bifunctional, *e.g.* 4-nitrophenol and 3-methylbenzoic acid, there may be inclusion of the same guest in more than one way, *i.e.*, competition of the two 'ends' of the guest molecule for the cyclodextrin cavity, necessitating the consideration of two or more simultaneous equilibria.

DISCUSSION

The thermodynamics of the binding of 4-nitrophenol to α-cyclodextrin determined by Cramer *et al.*[1] is given in Table 1. The agreement between their values and those reported here is good for K (and ΔG_b) but only fair for ΔH_b. However, the ΔH_b values determined here by direct measurement should be better than the value obtained by Cramer *et al.* from the temperature dependence of the equilibrium constant. The equilibrium constant found in this study for $NaClO_4$ binding to α-cyclodextrin is in good agreement with the value determined by Cramer *et al.*[1]

An equilibrium constant ($K = 200$) has been reported for the formation of a β-cyclodextrin–hydrocinnamic acid complex.[22] However, as noted in Table 2, no measurable interaction was detected here for β-cyclodextrin with hydrocinnamic acid. This is an indication that the complex is formed but that the enthalpy change for the reaction is very small.

Some of the previous studies [1,2] have investigated the binding of acid anions such as benzoate, phenolate, and hydrocinnamate to cyclodextrin in buffered solutions with pH values ranging from 9 to 13. In this pH range the cyclodextrin is also ionized to some extent since, like most sugars, it has secondary hydroxy-groups with pK values of *ca.* 12.[10,23] The application of a 1 : 1 reaction scheme to the calorimetric data for the reaction of α-cyclodextrin with NaOH resulted in a very small equilibrium constant ($K < 1$) and a very large enthalpy change (*ca.* −26 kcal mol^{-1}) which is obviously incorrect when compared with data for proton ionization of other sugars. The error is principally due to the assumption that only one reaction takes place. This is not unexpected since several secondary hydroxy-groups are present in the cyclodextrin molecule and these groups should have very similar pK values. These results do, however, indicate that any experiments done on the binding of cyclodextrins in solutions with pH values above 10 must take into account the fraction of the cyclodextrin which exists in an ionized anionic state. Calorimetric data on the reaction of α-cyclodextrin with NaOH and various sodium salts such as sodium benzoate, sodium phenolate, and sodium hydrocinnamate are given in ref. 17. If accurate values for the ionization constants of α-cyclodextrin become available these data can be used to calculate the thermodynamics of binding of these anions to α-cyclodextrin.

The ability of α-cyclodextrin to bind several inorganic anions has previously been observed. Cramer *et al.* noted the inhibitory effect of ClO_4^-, NO_3^-, and I^- on the catalysis of pyrophosphate cleavage by α-cyclodextrin; SO_4^{2-} and PO_4^{3-} did not seem to complex with the cyclodextrin since no inhibition was noted in the presence of these ions.[1] In the present study three perchlorates were found to react with α-cyclodextrin (see Table 1). The data further show that the cyclodextrin–perchlorate complexes also include the cation. This is shown by the fact that anilinium perchlorate, perchloric acid, and sodium perchlorate have significantly different apparent enthalpy and entropy changes for the reactions. Titration of α-cyclodextrin with NaCl however, did not indicate that any interaction took place. Thus, since Na^+ apparently does not bind to α-cyclodextrin in the absence of ClO_4^-, the only reasonable explanations are that α-cyclodextrin is binding the ion pair or that α-cyclodextrin binds ClO_4^- and this complex in turn binds the cation. It should be noted that the equilibrium constant for the binding of perchlorate appears to be independent of the cation because of the compensation of the changes in ΔH and ΔS, which occur when the cation is varied.

Table 3 shows a comparison of the ΔG_b and ΔH_b values for the interaction of four different guests with each of

TABLE 3

Comparison of the binding of selected guest molecules by α- and β-cyclodextrin

	Host			
	α-Cyclodextrin		β-Cyclodextrin	
Guest	$-\Delta G_b$/ kcal mol^{-1}	$-\Delta H_b$/ kcal mol^{-1}	$-\Delta G_b$/ kcal mol^{-1}	$-\Delta H_b$/ kcal mol^{-1}
(1)	5·7	1·8	4·6	2·6
(2)	4·1	9·6	2·9	7·6
(16)	2·9	7·3	4·1	10·5
(15)	3·3	11·6	9·6	11·7

the two cyclodextrin hosts and it appears that the binding of at least these four particular guests is generally quite similar for α- and β-cyclodextrin. β-Cyclodextrin binds 4-nitrophenol and 3-methylbenzoic acid more strongly than α-cyclodextrin but the unsubstituted benzoic acid and phenol are more strongly bound to α-cyclodextrin.

The ΔH_b values also follow the same general trends for the binding to the two host molecules. The simplest explanation of these observations is that the guests with the smaller molecular volumes fit the α-cavity best while those with larger molecular volumes fit the larger cavity of β-cyclodextrin better. A result that seems inexplicable, however, is that some guests which are similar to those listed in Table 3, *i.e.* 4-aminobenzoic acid and L-phenylalamine, were found to bind strongly and exothermically to α-cyclodextrin but did not interact measurably with β-cyclodextrin.

A plot of ΔG_b values *vs.* ΔS_b values for binding of the various guest molecules to α-cyclodextrin is given in Figure 2. With the exception of the perchlorates which were discussed earlier, L-tryptophan, 4-nitrophenol, and possibly indole, fall significantly off the line. It can be

[22] W. A. Pauli and J. L. Lach, *J. Pharm. Sci.*, 1965, **54**, 1745.
[23] R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *J. Amer. Chem. Soc.*, 1966, **88**, 2641.

seen in Figure 2 that the ΔG_b values change very little for the whole series of guest molecules. In effect, changes in ΔH_b are almost compensated for by changes in ΔS_b.

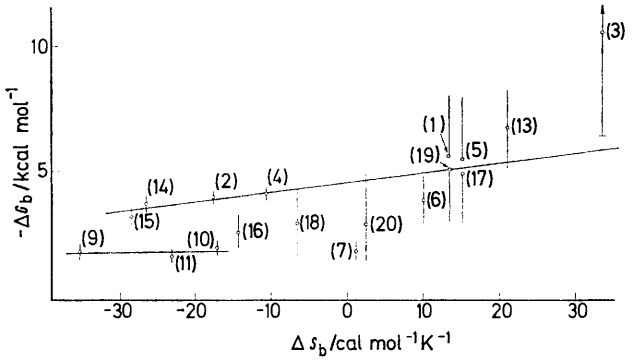

FIGURE 2 Plot of ΔG_b *vs.* ΔS_b for binding of various molecules to α-cyclodextrin. Vertical bars indicate error limits. Numbers refer to compounds in Table 1

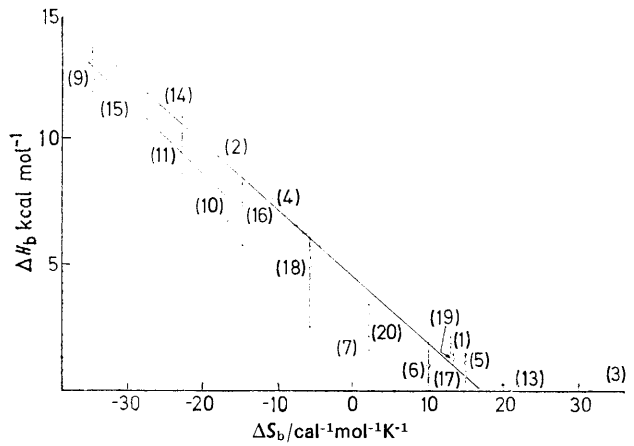

FIGURE 3 Plot of ΔH_b *vs.* ΔS_b for binding of various molecules to α-cyclodextrin. Vertical bars indicate error limits. Numbers refer to compounds in Table 1.

A plot of ΔH_b values *vs.* ΔS_b values is given in Figure 3. The slope of the line in this plot corresponds to the iso-equilibrium temperature [24] and has a value of 265 K. Since the temperature at which these experiments were done was only slightly higher than 265 K, it is evident that changes in ΔH will compensate for changes in ΔS to a large degree. This compensation effect has been observed frequently in water.[24] A reaction in which the solvent becomes more disordered will usually proceed with a favourable entropy change but the solvent disordering will be accompanied by the breaking of some solvent–solvent bonds which will result in an unfavourable enthalpy change. (This somewhat simplistic description of the compensation effect is obviously limited to highly ordered and hydrogen bonded solvents such as water.)

The thermodynamics of formation, in water, of the cyclodextrin complexes can be correlated by the principles given in the above discussion. For example, the large enthalpy change accompanying the binding of benzoic acid to α-cyclodextrin results from the process of forming hydrogen bonds both between the guest and the cyclodextrin and between the water molecules that become part of the bulk water during the reaction (*i.e.* water that was removed from the carboxy-group or displaced from the cyclodextrin cavity becomes bulk water and as such is hydrogen bonded). The net result must be a gain in the number of hydrogen bonds. The unfavourable entropy change is assumed to result from the greater ordering of the displaced water molecules in the bulk water structure. This picture is over simplified but does serve to indicate that the differences in the thermodynamics of binding of different guest molecules to cyclodextrins are largely the result of differences in the solvation of the guest molecules. It is apparent from this study as well as from previous work [1,2,4] that the cyclodextrins are not very discriminating hosts in water because of the compensation effect. The small range of equilibrium constants reported here and elsewhere (*e.g.* see ref. 4) show that the cyclodextrins bind a vast array of guest molecules equally well. If specificity of substrate binding is a necessary characteristic for a viable model for substrate binding to enzymes then the cyclodextrins fail to satisfy this criterion.

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[24] R. Lumry and S. Rajender, *Biopolymers*, 1970, **9**, 1125.