Chiral Host–Guest Complexes: Interaction of α-Cyclodextrin with Optically Active Benzene Derivatives

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The binding to α-cyclodextrin of a series of small, chiral benzene derivatives has been studied by direct reaction microcalorimetry and by a spectral competitive inhibition technique. A small, but distinct, chiral discrimination is demonstrated in the binding of the optical isomers of phenylalanine and α-methylbenzylamine, whereas mandelic acid, amphetamine, and phenyltrifluoroethanol show no such effect. The enthalpies of complex formation are remarkably similar for all compounds studied, ΔH *ca.* −3.5 kcal mol^{-1} (25 °C; pH 11.0; 0.1M-phosphate buffer). No obvious correlation of chiral effects with molecular structure has been found. It is concluded that insertion of the aromatic ring in the central cavity of α-cyclodextrin provides the major driving force for complex formation, and that chiral interactions external to the cavity are of minimal importance in this class of compounds.

CYCLODEXTRINS are torus-shaped cyclic oligosaccharides made up of α-1,4-linked D-glucopyranose units, produced in the breakdown of starch by *B. Macerans* amylase.[1] The non-polar central cavity of these molecules can accommodate a wide variety of organic and inorganic guest molecules, and inclusion complexes with α-cyclodextrin (α-CD, cyclohexa-amylose) and β-cyclodextrin (β-CD, cyclohepta-amylose) have been extensively studied.[1,2] In addition, cyclodextrins can catalyse (or, in some cases, inhibit) various reactions involving included guest molecules.[3] There seem to be few restrictions, other than size and polarity, on the type of guest molecule which can be incorporated into a cyclodextrin complex but, despite this rather broad structural specificity, some instances of stereospecific interactions have been described.[3] In particular, the intrinsic chirality of cyclodextrins is reflected both in the reactivity of guest molecules [3] and in their spectroscopic properties; [4] co-crystallization with α- or β-CD has resulted in partial resolution of racemic mixtures of a variety of optically active compounds.[5]

[1] D. French, *Adv. Carbohydrate Chem.*, 1957, **12**, 189.

[2] (*a*) F. Cramer, 'Einschlussverbindungen,' Springer–Verlag, Berlin 1954; (*b*) J. A. Thoma and L. Stewart in 'Starch: Chemistry and Technology,' eds. R. L. Whistler and E. F. Paschall, Academic Press, New York, 1965, vol. 1, ch. IX.

[3] (*a*) F. Cramer and H. Hettler, *Naturwiss.*, 1967, **54**, 625; (*b*) D. W. Griffiths and M. L. Bender, *Adv. Catalysis*, 1973, **23**, 209.

[4] See for example K. Takeo and T. Kuge, *Stärke*, 1972, **24**, 281 (o.r.d. and c.d.); D. D. MacNicol and D. S. Rycroft, *Tetrahedron Letters*, 1977, 2173 (n.m.r.).

[5] (*a*) F. Cramer and W. Dietsche, *Chem. Ber.*, 1959, **92**, 378; (*b*) H. P. Benschop and G. R. Van den Berg, *Chem. Comm.*, 1970, 1431; (*c*) M. Mikolajczyk, J. Drabowicz, and F. Cramer, *ibid.*, 1971, 317.

As part of a general study of host–guest interactions, we have investigated the possible enantiomeric discrimination in complexes of α-CD with a series of optically active, mononuclear aromatic compounds. The dissociation constants and enthalpies of complex formation in aqueous solution, determined by direct reaction calorimetry and by competition with *p*-nitrophenolate, are reported here.

EXPERIMENTAL

Calorimetry.—Heats of complex formation were determined using an LKB 2107-020 flow microcalorimeter [6] at 25 (±0.01) °C. Flow rates were in the region of 0.1 ml min^{-1} (determined accurately by weight for each experiment), and the calorimeter was calibrated both electrically and chemically, using the known heat of proton ionization of tris(hydroxymethyl)aminomethane.[7] Heats of complex formation were corrected for the appropriate heats of dilution, measured separately under identical conditions. In a typical experiment α-CD solution (4mM final concentration) was mixed in the flow calorimeter with a series of concentrations (2—60mM final concentration) of guest molecules, alternating the enantiomeric form so as to minimise systematic errors. Steady state heats of mixing, integrated over periods of at least 10 min to reduce signal-to-noise ratios, were typically in the range 0—4 μV on the calorimeter output, corresponding to 0.1 mcal min^{-1}, approximately.

Spectral Titration.—Solutions of *p*-nitrophenolate (*ca.* 5×10^{-5}M), with or without known concentrations of chiral competitors, were titrated with increasing concentrations of α-CD (0—1.5mM), and difference spectra over the range 330—520 nm were recorded at each addition (Pye–Unicam SP1800 dual-beam recording spectrophotometer, with thermostatted cells). The maximal change in nitrophenolate absorbance occurring at 370 nm was used for computing binding parameters. Difference spectra showed isosbestic points at 399 and 442 nm. consistent with simple 1 : 1 α-CD–nitrophenolate complex formation, and none of the chiral competitors gave absorbance in this spectral region. U.v. spectra (230—300 nm) were used to check purity and concentrations in each case.

Materials.—α-Cyclodextrin, phenylalanine (Sigma), mandelic acid (Aldrich), phenyltrifluoroethanol (Burdick and Jackson), and *p*-nitrophenol (B.D.H.) were used as received. Amphetamine (Emanuel) and α-methylbenzylamine (Aldrich) were redistilled before use. The specific rotation in water of the α-cyclodextrin (vacuum dried over P_2O_5) was +150.1° (lit.,[8] +150.5 ± 0.5°). $[\alpha]_D$ (H_2O) values for the optically active pairs were as follows: amphetamine +40°, −40°; mandelic acid +153°, −150°; α-methylbenzylamine +24°, −24°; phenylalanine +33.7°, −33.4°; phenyltrifluoroethanol +33°, −33°. Phosphate buffer (0.1M-Na_2HPO_4–NaOH), pH 11.0, made up in glass-distilled water, was used throughout for the calorimetric and spectroscopic procedures.

Analysis of Binding Data.—In the simple case of 1 : 1 complex formation between α-cyclodextrin (C) and guest molecule (G), $C + G \rightleftharpoons CG$ with dissociation constant $K_g = [C][G]/[CG]$, the relationship between concentration of complex and free guest concentration may be written as (1). The heat of mixing in a calorimetric experiment is $Q = \Delta H[CG]$, giving equation (2) where ΔH is the enthalpy of complex formation.

$$[C]_{Total}/[CG] = 1 + K_g/[G] \quad (1)$$

$$[C]_{Total}/Q = 1/\Delta H + K_g/\Delta H[G] \quad (2)$$

Calorimetric data were analysed according to this expression, and also the equivalent Benesi–Hildebrand equation, using a convergent iterative procedure to give best fit to the straight line plot of $1/Q$ *versus* $1/[G]$, yielding ΔH and K_g from the intercept and slope, respectively.

In the presence of a second guest, or inhibitor (I), which competes for the same binding site in cyclodextrin, the relevant expression is (3) where $K_I = [C][I]/[CI]$ is the

$$[G]_{Total}/[CG] = 1 + \frac{K_g}{[C]_{Total} - [CG]}\left(1 + \frac{[I]}{K_I}\right) \quad (3)$$

dissociation constant for the competing complex.

In the spectral titrations the change in absorbance, ΔA, of *p*-nitrophenolate at 370 nm when bound to α-CD [9] was used as a probe of α-CD–nitrophenolate complex concentration, $\Delta A = \Delta A_{max.}[CG]$, giving equation (4). Thus, as a first approximation, plots of $1/\Delta A$ *versus* $1/[C]_{Total}$ in the presence and absence of a known concentration of inhibitor, give straight lines with common intercept on the $1/\Delta A$ axis, the ratio of the slopes being determined by the extent of inhibition.

$$\frac{[G]_{Total}}{\Delta A} = \frac{1}{\Delta A_{max.}} + \frac{K_g}{\Delta A_{max.}([C]_{Total} - [CG])}\left(1 + \frac{[I]}{K_I}\right) \quad (4)$$

Once again the iterative procedure was used to give best least-squares fit to this, and to the equivalent Benesi–Hildebrand equation, to obtain the dissociation constants K(cyclodextrin–nitrophenolate) and K_I (cyclodextrin–inhibitor, *i.e.* chiral guest molecules).

RESULTS AND DISCUSSION

Double reciprocal plots of the spectral titration with α-CD of *p*-nitrophenolate in the presence and absence of the chiral inhibitors are shown in Figure 1 for four of the systems studied. (The results for D- and L-amphetamine are not shown, but they exhibit similar behaviour.) It is clear that (i) in all cases the chiral guest molecules compete for the same binding site on α-CD as *p*-nitrophenolate and (ii) in two cases (phenylalanine and α-methylbenzylamine) distinct differences are observed in the dissociation constants for the separate enantiomers. In the case of phenylalanine this chiral discrimination is confirmed by the direct thermal titration of α-CD with D- and L-phenylalanine (Figure 2). Unfortunately, thermal titrations with α-methylbenzylamine and amphetamine could not be done satisfactorily because of low solubility and adsorption of these compounds to the calorimeter flow tubing. In the cases where comparison is possible, however, dissociation constants obtained by the two techniques are in reasonable agreement, though those from calorimetric titrations are generally the less

[6] P. Monk and I. Wadsö, *Acta Chem. Scand.*, 1968, **22**, 1842.
[7] I. Grenthe, H. Ots, and O. Ginstrup, *Acta Chem. Scand.*, 1970, **24**, 1067.
[8] D. French, M. L. Levine, J. H. Pazur, and E. Norburg, *J. Amer. Chem. Soc.*, 1949, **71**, 353.
[9] F. Cramer, W. Saenger, and H-Ch. Spatz, *J. Amer. Chem. Soc.*, 1967, **89**, 14.

accurate because of the weak association and relatively low enthalpies of complex formation. K_{diss} for α-CD–*p*-nitrophenolate (Table) agrees well with previous determinations.[9,10]

clear whether the differences in equilibrium properties of the enantiomeric complexes with phenylalanine or α-methylbenzylamine are due specifically to enthalpic or to entropic effects.

Thermodynamics of binding of chiral guest molecules to α-cyclodextrin [a]

Guest	Enantiomeric form	10^3K_{diss}/l mol^{-1}		$-\Delta H$/kcal mol^{-1} [c,d]
Phenylalanine	D	48.5 (±1.3) [b]	55.3 (±3.7) [c]	3.9 (±0.2)
	L	63.0 (±4.5)	64.6 (±0.9)	3.7 (±0.1)
α-Methylbenzylamine	+	38.5 (±2.8)		3.6 (±0.8)
	−	29.8 (±1.5)		3.7 (±0.3)
Mandelate	D	126 (±19)	129 (±32)	3.1 (±0.6)
	L	121 (±10)	129 (±26)	3.4 (±0.5)
Phenyltrifluoroethanol	D	17.6 (±1.5)	24.9 (±4.8)	3.5 (±0.5)
	L	17.5 (±1.5)	19.6 (±6.9)	3.0 (±0.7)
Amphetamine	D	41.8 (±1.0)		3.0 (±0.5)
	L	39.6 (±1.7)		3.0 (±0.5)
p-Nitrophenolate		0.53 (±0.02)		

[a] 25 °C, pH 11.0, 0.1M-phosphate buffer. Figures in parentheses are standard deviations estimated from least-squares analysis. [b] Obtained from spectral inhibition titrations. [c] Obtained from calorimetric titrations. [d] 1 cal = 4.184 J.

Although we have observed two cases of chiral discrimination the effect is quite small, the differences

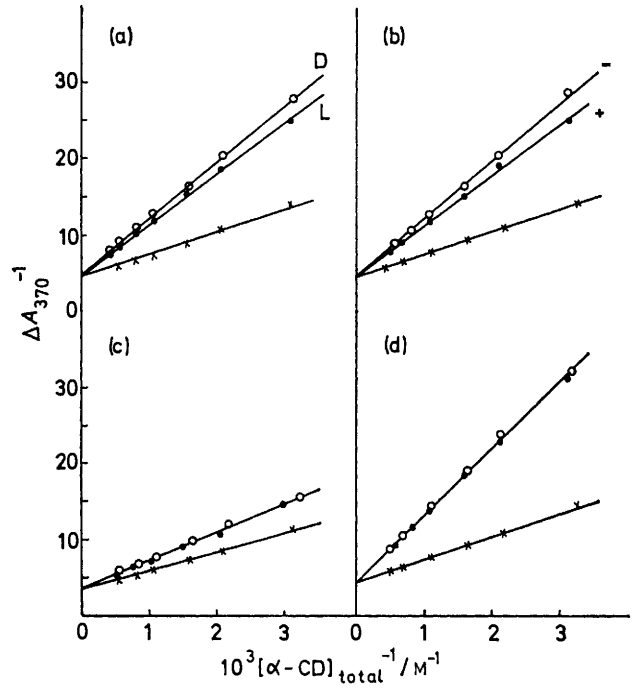

FIGURE 1 Double-reciprocal plots of the spectral titration of *p*-nitrophenolate (5—6 × 10^{-5}M) with α-cyclodextrin, in the presence (○, ●) or absence (×) of competing enantiomers (25 °C; pH 11.0; 0.1M-phosphate): (a) phenylalanine, 0.061M; (b) α-methylbenzylamine, 0.046M; (c) mandelate, 0.053M; (d) phenyltrifluoroethanol, 0.034M

amounting to only *ca.* 0.2 kcal mol^{-1} in free energy of complex formation, and is clearly not a general phenomenon for this class of guest molecules. Overall the free energies of complex formation range from *ca.* −1.2 to −2.4 kcal mol^{-1}, whereas the enthalpies of formation cluster around −3.5 kcal mol^{-1}. Because of experimental uncertainties in the estimates of $\Delta H°$ it is not

[10] R. J. Bergeron, M. A. Channing, G. J. Gibeily, and D. M. Pillor, *J. Amer. Chem. Soc.*, 1977, **99**, 5146.

By analogy with other cyclodextrin inclusion complexes the most likely mode of binding of these monosubstituted benzene molecules is by insertion of the benzene ring into the cyclodextrin central cavity. In the case of phenylalanine this seems to be supported by recent ^{1}H n.m.r. studies.[11] Manipulation of space-filling models indicates that in such complexes the substituent groups would be largely exposed to solvent, though a variety of interactions with the rim of the cyclodextrin torus is feasible. It is at this level that enantiomeric differences would be manifest. The apparent insensitivity of the enthalpy of complex formation to the nature, or chirality, of the substituent groups, together with small or non-existent enantiomeric effects of K_{diss},

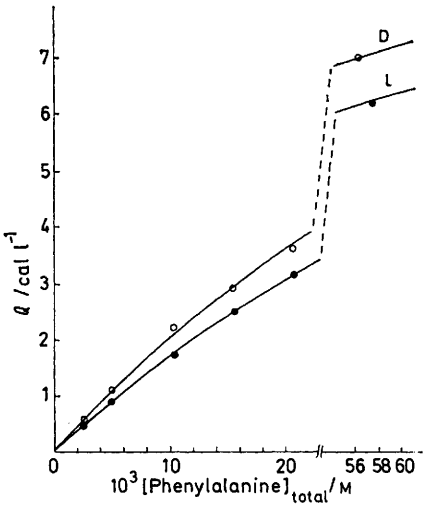

FIGURE 2 Heat effects, corrected for heats of dilution, upon mixing α-cyclodextrin (3.66 × 10^{-3}M final concentration) with various concentrations of D- or L-phenylalanine (25 °C; pH 11.0; 0.1M phosphate). Solid lines are theoretical thermal titration curves computed using the parameters in the Table

suggest that interactions in the cyclodextrin cavity provide the major driving force for complex formation

[11] D. J. Wood, F. E. Hruska, and W. Saenger, *J. Amer. Chem. Soc.*, 1977, **99**, 1735.

and that contributions from interactions outside the central cavity are minimal. This seems reasonable from examination of molecular models since no severe steric hindrances are found, and hydrogen bonds or other polar interactions with the cyclodextrin rim would be inhibited by exposure to the aqueous environment.[12]

[12] (*a*) I. M. Klotz and J. S. Franzen, *J. Amer. Chem. Soc.*, 1962, **84**, 3461; (*b*) I. M. Klotz and S. B. Farnham, *Biochemistry*, 1968, **7**, 3879.

It should be noted that these observations on the equilibrium thermodynamics of chiral complexes do not preclude more marked stereoselective effects in non-equilibrium properties such as chemical reactivity.

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