A Novel Assembly of Cyclodextrins with 4,4′,4″,4‴-(21*H*,23*H*-Porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) through Hydrogen Bonds

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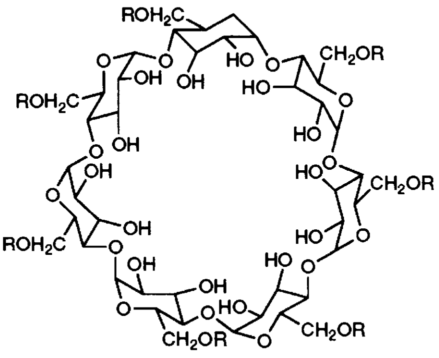
Semi-closed assemblies are formed from cyclodextrins (CyDs) and 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis benzoic acid (PTTKBA); the assemblies are a new group of host molecules that possess inclusion properties different from those of the component CyDs.

Cyclodextrins (CyDs, Fig. 1) include partially or totally[1,2] hydrophobic organic compounds and distribute these complexes in aqueous systems,[1–4] finding use in industrial, environmental and pharmaceutical applications including drug solubilisation.

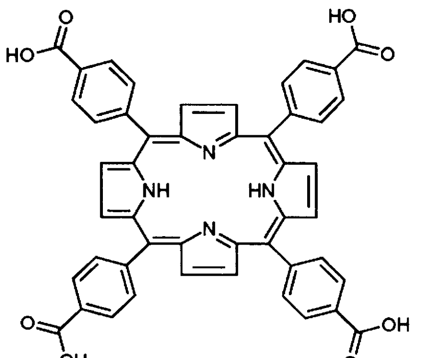
The open structure of a CyD may form a semi-closed or closed assembly by capping one or both of the cavity edges with a 'cap' molecule. These assemblies may have significantly different properties to the component CyDs. Since CyDs possess hydroxy groups positioned on both edges of their cavity; a cap molecule could easily be attached to the two edges through hydrogen bonds. The ideal cap molecule should be flat and round to fit the CyD. The hydrogen-bonding groups on the cap molecule should also be positioned along the round edge uniformly. 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,20-tetrayl)tetrakis benzoic acid (PTTKBA, Fig. 1) is a potential choice since it consists of a planar porphine ring edged with four benzoxy groups, which could form hydrogen bond to the CyD hydroxy groups.

Hydrogen bonding between CyDs and PTTKBA was examined first by spectrophotometry and fluorometry. The formation of the hydrogen bonds was expected to have a strong effect on the absorption and fluorescence characteristics of PTTKBA since the hydrogen bonds are conjugated to the porphine ring through the benzyl ring. The effect of the complexation with hp-β-CyD on the absorption spectrum of PTTKBA is illustrated in Fig. 2 where the absorption maximum at 414 nm shifts to longer wavelengths in the presence of hp-β-CyD. In the fluorescent spectrum of PTTKBA the complex formation enhanced the fluorescence by *ca.* 100% with excitation at 395 and emission at 650 nm, but the wavelength of maximum emission shifted only slightly from 648 to 652 nm.

A CyD molecule possesses hydroxys on both edges and might be able to combine two PTTKBA molecules. However, based on the curves of spectral shift *vs.* mole ratio in Fig. 3, the molar ratio in the CyD–PTTKBA complexes is one to one, indicating the formation of a semi-closed assembly. Similarly,



1 β-CyD: R = H
hp-βCyD: R = CH$_2$CHOHMe



2 PTTBKBA

Fig. 1 Structures of CyDs and PTTKBA

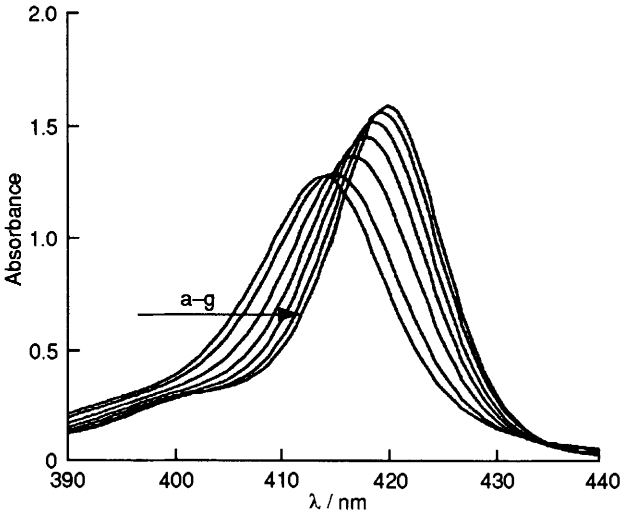


Fig. 2 Effect of hp-β-CyD on the absorption spectrum of PTTKBA in pH 7.0, 0.1 mol dm^{-3} potassium phosphate solution. [CyD] : [PTTKBA] (μmol dm^{-3} : μmol dm^{-3}): (*a*) 0 : 5; (*b*) 0.5 : 5; (*c*) 1.5 : 5; (*d*) 2.5 : 5; (*e*) 3.5 : 5; (*f*) 5 : 5; (*g*) 7.5 : 5.

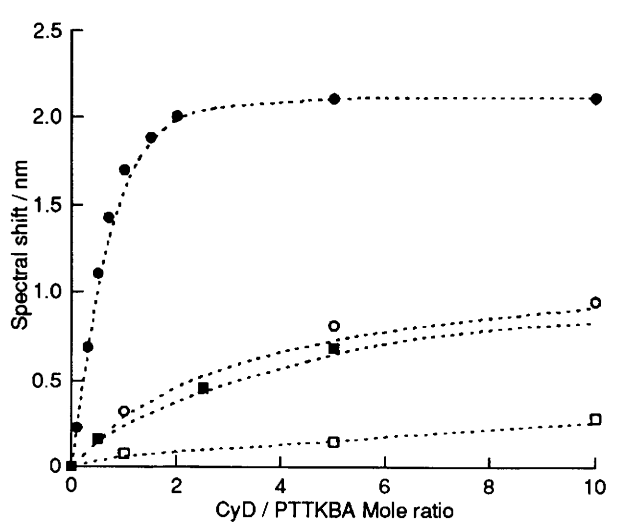


Fig. 3 Plots of spectral shift *vs.* molar ratio between CyDs and PTTKBA. (□) α-CyD; (■) β-CyD; (○) γ-CyD; (●) hp-β-CyD

plotting fluorescent intensity *vs.* mole ratio also showed a complexation ratio of one to one. Fig. 4 schematically represents the formation of a semi-closed assembly between CyDs and PTTKBA. The discussion that follows will explain why the formation ratio is one to one and to which of the two edges of CyDs the cap molecule is bonded. According to Sandarajam and Rao,[5] the secondary hydroxys, C′-2 and C′-3, form strong intramolecular hydrogen bonds. These intramolecular hydrogen bonds could reduce the formation enthalpy of an assembly by about 25 kcal mol^{-1}. In addition, since the sizes of the two component molecules may not spatially fit one another entirely and, furthermore, the frames of the two component molecules are rigid, the flexibility of the bonding groups on CyDs is also an important factor affecting the formation of an assembly. The primary hydroxys are more flexible than the secondary hydroxys and, therefore, can adjust their position more freely toward and approaching the benzoxy groups on PTTKBA. Considering the reduced formation enthalpy and limited flexibility of the secondary hydroxys leads to the reasonable conclusion that the primary hydroxys on CyDs are essentially more favourable to form hydrogen bonds with the benzoxys than the secondary hydroxys. This conclusion is also supported by the observation that hp-β-CyD containing the flexible hydroxypropyl groups on the edge of the primary hydroxys forms the most stable hydrogen-bond complex with PTTKBA (Fig. 3).

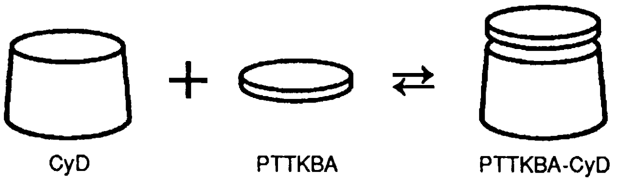


Fig. 4 Schematic for the formation of a semi-closed assembly between CyDs and PTTKBA

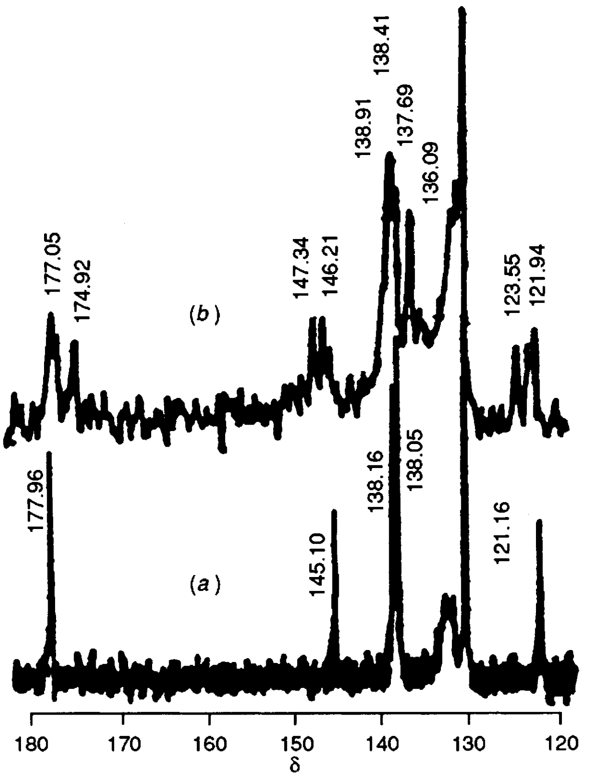


Fig. 5 ^{13}C NMR spectra of PTTKBA and its complex with hp-β-CyD. Proton decoupled; frequency: 75.4 MHz; about 4000 scans. (*a*) 50 mmol dm^{-3} PTTKBA and (*b*) 50 mmol dm^{-3} PTTKBA and 50 mmol dm^{-3} hp-β-CyD in 0.1 mol dm^{-3}, pH 7.0 phosphate aqueous solution.

Further evidence for the semi-closed assembly was observed in ^{13}C NMR measurement of the hp-β-CyD-PTTKBA assembly. Hp-β-CyD forms a very stable complex with PTTKBA and possesses large solubility in water, facilitating the ^{13}C NMR measurement. The NMR spectra of simple PTTKBA and its assembly with hp-β-CyD are shown in Fig. 5. The peaks of PTTKBA [Fig. 5(*a*)] appeared as two equi-intensive peaks with downfield or upfield shifts in the spectrum of the assembly [Fig. 5(*b*)]. For instance, the peak of the carboxy carbon at δ 177.96 appeared as two peaks at δ 177.05 and 174.92 obviously because of the shielding effect of the hydroxy proton. This observation of peak separation and shift thus provides a convincing evidence about the binding of PTTKBA to the top of hp-β-CyD through hydrogen bonds. The angular spacings of the hydrogen-bonding groups in the two molecules are different (90° for the carboxyls in PTTKBA and 51.4° for the hydroxys in hp-β-CyD). When PTTKBA is bonded to the top of hp-β-CyD with the four carboxylic groups of the former positioning towards four of the seven hydroxy groups of the latter, two of the carboxys are at 6.4° and the other two are at 19.3° relative to the hydroxys with which they most possibly form hydrogen bonds. This difference in relative angle means difference in strength of hydrogen bond and in chemical shift and, consequently, results in separation of the relevant ^{13}C peaks for simple PTTKBA into two equi-intensive peaks for the assembly.

Formation constants were estimated using the Benesi and Hildebrand eqn (1):[6]

$$\frac{[\mathrm{C_A}]_t}{d}\, l = \frac{1}{\alpha} + \frac{1}{K\alpha[\mathrm{C_D}]_t} \qquad (1)$$

where $[\mathrm{C_A}]_t$ is the total concentration of the donor; l is the path length of the cuvette; d is the change in absorption characteristics; α is a constant proportional to the change; K is the formation constant of the complex of interest; $[\mathrm{C_D}]_t$ is the total concentration of the acceptor. At a constant concentration of PTTKBA ($[\mathrm{C_A}]_t$), all plots of $[\mathrm{C_A}]_t l/d$ *vs.* $1/[\mathrm{C_D}]_t$ were linear with an intercept ($1/\alpha$) at the $[\mathrm{C_A}]_t l/d$ coordinate. The estimated formation constants are 8.1×10^3 dm^3 mol^{-1} for α-CyD, 7.0×10^4 dm^3 mol^{-1} for β-CyD, 5.2×10^4 dm^3 mol^{-1} for γ-CyD, and 6.9×10^5 dm^3 mol^{-1} for hp-β-CyD, respectively.

The authors appreciate the efforts of Dr F. Sauriol of the Department of Chemistry, McGill University, in performing the NMR measurements.

Received, 29th April 1994; Com. 4/02555E

References

1 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, 1978.
2 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344.
3 F. Cramer and H. Hettler, *Naturwissenschaft*, 1967, **54**, 625.
4 J. A. Thoma and L. Stewart, *Starch: Chemistry and Technology*, ed. R. F. Whistler and E. F. Pashall, vol. 1, Academic, New York, 1965.
5 P. R. Sundararajan and V. S. Rao, *Carbohydr. Res.*, 1970, **13**, 351.
6 H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.