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METHYL SALICYLATE FLUORESCENCE AS A PROBE OF THE GEOMETRY OF COMPLEXATION TO CYCLODEXTRINS

G. SIDNEY COX and NICHOLAS J. TURRO*
Department of Chemistry, Columbia University, New York, NY 10027, USA

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Abstract—Methyl salicylate (MS) is found to form complexes with α-, β- and γ-cyclodextrins in aqueous solution. The fluorescence of the complexes differ dramatically from that of MS in pure aqueous solution in the case of α- and γ-cyclodextrin, but is similar to that of MS in pure aqueous solution in the case of β-cyclodextrin. The binding constants for the complexes have been evaluated and the fluorescence of the complexes has been employed to infer the geometry of the complexes.

INTRODUCTION

Cyclodextrins form 1:1 complexes with many organic substrates in aqueous solution (Bender and Komiyama, 1978). It is generally assumed that the substrate molecule binds inside the hydrophobic cavity of the cyclodextrin; however, in many cases the detailed structure of the complex is not known. X-ray crystal structure data of crystalline complexes is the most direct method for determining complex geometry, but it has only been achieved in a limited number of cases (Harata *et al.*, 1982; Hursthouse *et al.*, 1982). Several indirect methods are also available, such as NMR, induced circular dichroism and relative rates of ester hydrolysis. Nuclear magnetic resonance can indicate binding of aromatic molecules into the interior cavity by observation of larger upfield shifts of the interior cyclodextrin hydrogens compared to the exterior hydrogens (Turro *et al.*, 1982a; Burgeron and Burton, 1982). More exact geometries can be determined with NMR, by observation of specific intermolecular nuclear Overhauser enhancements between substrate and cyclodextrin. However, this has been observed in very few cases (Bergeron *et al.*, 1978; Bergeron and Rowan, 1976). Nuclear magnetic resonance is not always possible, since high concentrations of substrates are needed, which can prove to be difficult conditions to achieve for organic molecules with low water solubilities.

The complexation of achiral substrates to cyclodextrins which are chiral can lead to induced circular dichroism in the UV-VIS absorption bands of the substrate (Shimizu *et al.*, 1981; Shimizu *et al.*, 1982). The sign of the circular dichroic effects can sometimes be used to determine the direction of the substrate dipole relative to the axis of the cyclodextrin (Schipper and Rodger, 1983). The relative rates of ester hydrolysis of closely related esters has been used to estimate binding geometries (Griffiths and Bender, 1973). However, one must keep in mind the Curtin–Hammett principle before any structural interpretation can be made (Seaman, 1983).

Since the fluorescent properties of many molecules are highly dependent on environment (Turro *et al.*, 1980), fluorescence is potentially a useful method for determining complexation geometry. Several reported examples of the use of fluorescence have demonstrated that organic substrates can observe an environment less polar than water when bound to cyclodextrins (Cramer *et al.*, 1967; Turro *et al.*, 1982b; Yorozu *et al.*, 1982). We recently demonstrated that dimethylaminobenzonitrile is in a much less polar environment when bound to α-cyclodextrin compared to β-CD. The result could indicate totally different binding geometries, but if the exact nature (and location) of the polarity effect is not known, then interpretation of fluorescence to indicate complexation geometries is difficult.

The fluorescence of 2-naphthol has been used recently as a probe for geometry of complexation to cyclodextrins (Yorozu *et al.*, 1982). The results indicated that the hydroxy group of 2-naphthol is buried inside the cavity of the cyclodextrin for α- and β-CD, but for γ-CD, the hydroxy group was observing an aqueous environment. We wish to report the flourescence properties of methyl salicylate in the presence of cyclodextrins and show how interpretations of changes in fluorescence, induced by complex formation, allows the deduction of complexation geometries.

MATERIALS AND METHODS

Methyl salicylate (99+%, Gold Label), α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin were obtained from Aldrich Co. and used as received. Distilled water and spectrograde solvents were used for all experiments. All aqueous solutions were freshly prepared, because methyl salicylate (MS)† hydrolyses to the acid which fluoresces at

*To whom correspondence should be addressed.

†*Abbreviations*: CD, cyclodextrins; k_{as}, association constant; LWE, long wavelength emission; MS, methyl salicylate; SWE, short wavelength emission.

410 nm with a higher quantum yield than the ester. Highly concentrated cyclodextrin samples were millipore filtered (Rainin, 0.5 μm cellulose) before use.

Ultraviolet absorption spectra were measured on a Perkin–Elmer 559-A spectrophotometer. Corrected fluorescence spectra were obtained on a SLM 4800A fluorescence spectrophotometer. All other fluorescence spectra were obtained on a Perkin–Elmer L5-5 fluorescence spectrophotomoter with data handling on the Perkin–Elmer data station. Equilibrium constants were determined by measuring the fluorescence intensity of MS at 450 nm at different cyclodextrin concentrations and analysing the data based on a modified Benesi–Hildebrand equation (Benesi and Hildebrand, 1949; Kinoshita *et al.*, 1974). For measurement of the fluorescence properties of MSA in cyclodextrin solutions, the concentration of cyclodextrin was adjusted so that 70% of the MS was in the complexed form.

RESULTS

Methyl salicylate (MS) exhibits dual fluorescence in most organic solvents (Klopffer and Naundorf, 1974; Sandros, 1976). The 450 nm emission has been assigned to a zwitterion excited state which is generated by intramolecular hydrogen transfer in conformer I (see Scheme I). Conformers II and III fluoresce at a shorter wavelength with a different excitation spectra from the emission at 450 nm. These results indicate that the fluorescence of MS is dependent on ground state conformation and that there is no excited state equilibrium between the three conformers (Smith and Kaufman, 1978; Ford *et al.*, 1980).

Table 1. Absorption and emission properties of methyl salicylate. aqueous cyclodextrins*

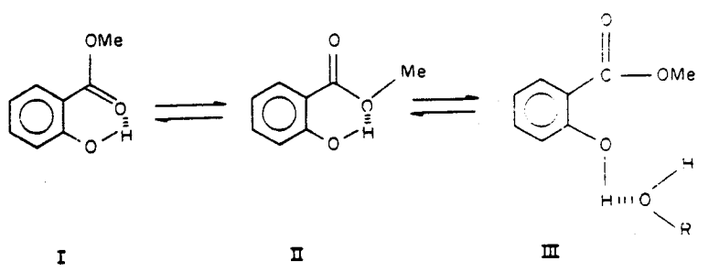
Cyclodextrin	$\lambda^{UV}{}_{max}$† UV (nm)	$\lambda^{F}{}_{max}$‡ SWE (nm)	LWE/SWE§
None	303	362	0.52
α-Cyclodextrin	307	339	0.93
β-Cyclodextrin	306	355	0.59
γ-Cyclodextrin	303	364	1.86

*In all cases, 70% of MS is complexed to the cyclodextrins. †λ_{max} of UV absorption. ‡λ_{max} of short wavelength fluorescence corrected for instrument response. §Ratio of fluorescence intensity of the long wavelength emission to the short wavelength emission corrected for emission due to MS not complexed to cyclodextrins.

Table 2. Equilibrium constants for complexation of methyl salicylate with cyclodextrins

Cyclodextrins	k_{as}* (M^{-1})
α-Cyclodextrin	20
β-Cyclodextrin	156
γ-Cyclodextrin	47

*Values were measured at 25 ± 0.5°C.



I II III

Scheme 1.

The fluorescence spectrum of MS changes quite dramatically with the H-bonding ability of the solvent (Table 3). With increasing H-bonding ability of the solvent, conformer III is favored over conformers I and II. This change in ground state conformation produces two effects on the fluorescence: (1) an increase in the intensity of short wavelength (SWE) to long wavelength emission (LWE), and (2) an increase in the $\lambda^{F}{}_{max}$ of the (SWE) from 330 nm (conformer II) to 360 nm (conformer III). The change of the $\lambda^{UV}{}_{max}$ is also indicative of solvent H-bonding to the solute. Therefore, there are three spectroscopic parameters of methyl salicylate which when measured are indicative of ground-state conformation.

The fluorescence of MS in aqueous solution changes with addition of α-, β-, and γ-cyclodextrins.

Table 3. Absorption and emission properties of methyl salicylate in organic solvents

Solvent	$\lambda^{UV}{}_{max}$*	$\lambda^{F}{}_{max}$†	Ratio of fluorescence intensity‡
Water	303	362	0.52
Methanol	305	350	0.60
Ethanol	306	349	1.12
2-Propanol	307	347	1.55
Ether	307	331	2.05

*λ_{max} of UV-VIS absorption in nm. †λ_{max} of short wavelength emission (SWE) in nm corrected for instrument response. ‡Ratio of fluorescence intensity of the long wavelength emission to the short wavelength emission.

Based on these fluorescence changes, equilibrium constants were calculated for each cyclodextrin (Table 2). The relative strength of the association constants correlates with the expected values based on the size of MS and the various cyclodextrin cavity sizes.

The fluorescence spectrum of MS in cyclodextrin solutions is dramatically different from the spectrum in water (Figs. 1–3). The spectrum for each MS/cyclodextrin solution is also different for each cyclodextrin. Table 1 lists the various emission and absorption properties of MS for each cyclodextrin.

In the case of α-cyclodextrin all three properties [λ^{UV}_{max}, λ^{F}_{max} (SWE), and LWE/SWE intensities] indicate large changes in the ground state conformation of MS when it is bound to α-CD. The results are consistent with the conclusions that conformers **I** and **II** are more favored in α-CD complex than in water. In particular, the λ^{F}_{max} of 339 nm is strongly suggestive of a small contribution from **III** to the population of the fluorescing ensemble. Whether these conformation changes are due to the necessity of packing into the α-CD cavity, or are due to decreased H-bonding to the solvent, is not known. In either case the result strongly suggests that the ester and hydroxyl groups are buried in the interior of the cyclodextrin cavity (Fig. 4). Inspection of molecular models indicate if the ester and hydroxyl groups are in the CD cavity, then the phenyl end protrudes into the aqueous layer. If the phenyl group was bound by the α-CD cavity, the ester and hydroxyl groups would be exposed to the aqueous phase, and conformer **III** should be present in significant amount. One might expect the MS would not bind strongly to α-CD by the geometry proposed here. This is supported by the relatively small equilibrium constant for binding (Table 2).

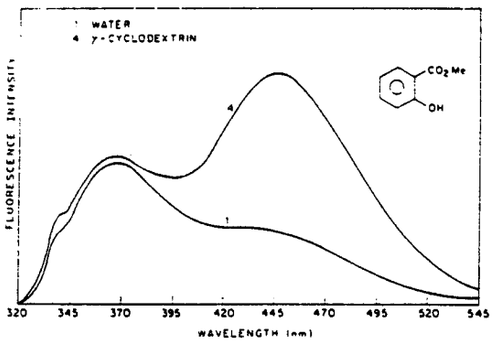


Figure 3. Total fluorescence spectrum of methyl salicylate in aqueous solution (curve 1) and in aqueous solutions containing γ-cyclodextrin.

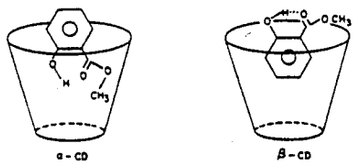


Figure 4. Favored geometries of fluorescing species deduced from fluorescence properties. See text for discussion.

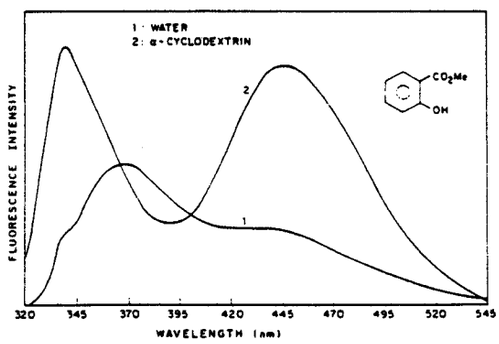


Figure 1. Total fluorescence spectrum of methyl salicylate in aqueous solution (curve 1) and in aqueous solution containing α-cyclodextrin.

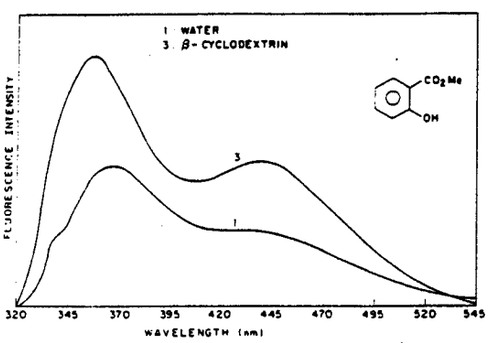


Figure 2. Total fluorescence spectrum of methyl salicylate in aqueous solution (curve 1) and in aqueous solution containing β-cyclodextrin.

In the case of β-cyclodextrin, conformers **I** and **II** are also favored relative to aqueous solution upon binding to the cyclodextrin as indicated by all three parameters (Table 1). However, the effect is smaller than was observed for α-CD. Therefore, a model of binding where the ester and hydroxyl groups enter the cavity first is inconsistent with the result. A more likely possibility which is supported by molecular models is a geometry where the phenyl is bound by the cavity, and the functional groups are only partially protected from the solvent (Fig. 4). Models suggest better binding in this geometry, a conclusion supported by the larger equilibrium constant for complexation to β-CD (Table 2).

In the γ-cyclodextrin case, the results are less clear cut. The λ^{UV}_{max} and λ^{F}_{max} (SWE) indicate no change in ground state conformation. However, the change in the LWE/SWE intensity ratio relative to water is more dramatic than for α- and β-CD. One

explanation for this result is that the ground state conformation is the same as in water, but that the zwitterionic excited state observes a non-polar environment. It is known that the non-radiative rate for this excited state increases with polarity (Smith and Kaufman, 1981). This effect causes a decrease in the quantum yield of the 450 nm emission with increasing polarity. The geometry of complexation of MS to γ-CD cannot be determined from the fluorescence and UV data, but the results illustrate two important points in interpreting this type of data: (1) It is very important to understand as completely as possible the many possible environmental effects on fluorescence, and (2) the various effects which environment can have on fluorescence make it important to compare several different properties. In this study the complication of polarity effects on fluorescence made it important to be able to compare three properties of MS. H-bonding affects all three properties, but polarity should affect only the LWE/SWE ratio.

CONCLUSION

The results presented here indicate the MS binds to α- and β-CD in different geometries. The favored geometries determined by the fluorescent results could not have been predicted based on inspection of molecular models. Attempts to support the complexation geometries by other techniques proved unsuccessful. The low solubility of MS in water and the weak equilibrium constants prevent studies of the effect of MS on the NMR of the CD's. Circular dichroism spectra were observed for MS in the presence of both α- and β-CD; however, the size of the induced circular dichroism was so small that no interpretation of the data was possible. Although the use of fluorescence to determine binding geometries to CD's is dependent on a substrate whose fluorescence changes with environment, this method should be useful for supplanting or supporting other indirect methods of structure determinations.

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