MICROENVIRONMENTAL EFFECTS OF CYCLODEXTRINS I: ENHANCEMENT OF INTRAMOLECULAR EXCIMER FORMATION OF POLYMETHYLENE-BIS-β-NAPHTHOATE VIA INCLUSION WITH CYCLODEXTRINS

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Summary

The effects of α -, β - and γ -cyclodextrins (CDs) on the fluorescence spectra of a series of polymethylene-bis- β -naphthoates (B_n) have been studied. It is observed that β -CD and γ -CD enhance B_n intramolecular excimer fluorescence, indicating the formation of two-to-one guest host inclusion complexes. The possible conformation of these inclusion complexes is discussed.

1. Introduction

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides consisting of six or more glucose units, and are shaped like doughnuts with hydrophobic cavities [1]. The most commonly used cyclodextrins are α -, β - and γ -CDs consisting of six units, seven units and eight units with cavity diameters of 4.5 Å, 7.0 Å and 8.5 Å respectively. The remarkable property of the CDs is their ability to form inclusion complexes with a variety of molecules provided that they fit entirely or at least partially into the CD cavity. Because of this substrate selectivity, CDs are often used as models for studying enzyme reactivity (for a review, see ref. 2). Typically, one-to-one guest host complexes are formed with α - and β -CDs. However, recent studies have shown that one γ -CD molecule can accommodate two aromatic molecules [3-6].

Luminescence probes which are included in the cavity of a CD may undergo characteristic changes in their luminescence intensity, spectral distribution and decay time, and therefore they are suited for the investigation of the complex structure dependence and dynamics of the inclusion process. Many excited aromatic chromophores form excimers which show a structureless red-shifted emission in addition to the normal monomer fluorescence. Bichromophoric compounds can form intramolecular excimers [7-11]. In our previous paper [12] we reported that polymethylene-bis- β naphthoates (B_n) in organic solvents exhibit very weak excimer emission compared with bis- β -naphthylalkanes [13]. We show here that B_n may form two-to-one guest host complexes with β -CD and γ -CD and that, as a result, the excimer fluorescence is markedly enhanced.



 B_2 , n = 2; B_3 , n = 3; B_4 , n = 4; B_5 , n = 5; B_{10} , n = 10.

2. Materials

The B_n were prepared according to the procedure described in a previous paper [12]. The CDs (Aldrich) were used as supplied. Sodium nitrite was recrystallized twice from doubly distilled water.

3. Methods

Saturated aqueous solutions of the B_n were made up and stirred with the CD for at least 2 d. The solutions were passed through millipore filters and then flushed with nitrogen for about 20 min. Uncorrected fluorescence spectra were recorded using a Hitachi MPF-4 spectrofluorometer using 280 nm as the excitation wavelength. The concentration of the probe was determined from the absorption of the filtered solution.

4. Results

4.1. Fluorescence spectra of complexes of B_n with CDs

The fluorescence spectra of $B_2 - B_{10}$ (5 × 10⁻⁶ M) in aqueous solution containing CDs (5 × 10⁻³ M) are shown in Figs. 1 - 5. Only very weak fluorescence can be detected in the absence of CDs. The intensity of the monomer fluorescence ($\lambda_{max} = 360$ nm) of B_4 , B_5 and B_{10} increases on adding 5 × 10⁻³ M α -CD, while the fluorescence intensity of B_2 and B_3 shows no obvious change.

Adding β -CD (5 × 10⁻³ M) to an aqueous solution of a B_n results in a strong structureless emission with $\lambda_{max} = 420$ nm. The excitation spectra at $\lambda_e = 420$ nm are identical with those at $\lambda_e = 360$ nm. Under the same conditions, ethyl β -naphthoate (concentration, below 5 × 10⁻⁶ M) shows no emission at $\lambda_{max} = 420$ nm. Thus, we attribute the new emission band to intramolecular excimer fluorescence. For the β -CD-B₂ complex the ratio I_E/I_M of excimer to monomer fluorescence intensity equals 2.7. The emission of β -CD-B₃ and β -CD-B₄ complexes is due almost entirely to the excimer



Fig. 1. Fluorescence spectra of B_2 in aqueous solution in the presence of 5×10^{-3} M CD. Fig. 2. Fluorescence spectra of B_3 in aqueous solution in the presence of 5×10^{-3} M CD.



Fig. 3. Fluorescence spectra of B_4 in aqueous solution in the presence of 5×10^{-3} M CD.

Fig. 4. Fluorescence spectra of B_5 in aqueous solution in the presence of 5×10^{-3} M CD.

 $(I_{\rm E}/I_{\rm M} > 10)$. However, for β -CD-B₅ and β -CD-B₁₀ complexes, $I_{\rm E}/I_{\rm M}$ is about 0.6.

 γ -CD-B_n complexes show only excimer emission, with the exception of γ -CD-B₂ ($I_{\rm E}/I_{\rm M} = 5$). It is interesting to note that γ -CD-B_n complexes emit with $\lambda_{\rm max} = 400$ nm which is quite different from the emission of β -CD-B_n complexes with $\lambda_{\rm max} = 420$ nm.



Fig. 6. γ -CD concentration dependence of B₅ fluorescence spectra in γ -CD aqueous solution.

4.2. The effect of CD concentration

The effect of γ -CD concentration on the fluorescence spectra of B₅ in aqueous solution is shown in Fig. 6. Similar results are observed for other members of the B_n series. Obviously, $I_{\rm E}/I_{\rm M}$ decreases as the γ -CD concentration increases.

4.3. Quenching of B_n fluorescence by sodium nitrite

In aqueous solution the fluorescence of B_n is easily quenched by sodium nitrite. For example, adding NaNO₂ in a concentration higher than 10^{-4} M is sufficient to quench the fluorescence of B₃ completely. However, association of the probes with the CD inhibits the quenching very effectively (an alternative explanation could be the inclusion of the nitrite ion in the CD cavity; see ref. 7, p. 350), *i.e.* emission is still observable from B₃ in aqueous solution containing 5×10^{-3} M γ -CD even in the presence of $5 \times$ 10^{-3} M NaNO₂. In order to quench completely the emission of B₃ in aqueous solution containing 5×10^{-3} M β -CD, 10^{-3} M NaNO₂ is required.

4.4. Quenching by oxygen

The effectiveness of O_2 quenching of monomer and excimer emission of B_5 in aqueous solution containing γ -CD and β -CD (5×10^{-3} M) are summarized in Table 1. I_{N_2} , I_{air} and I_{O_2} refer to the fluorescence intensity under nitrogen, air and oxygen purging respectively.

5. Discussion

The probes included in a CD cavity are protected from the dynamic bimolecular quenching process, and thus the quantum yield of fluorescence

TABLE 1

Emission maximum ^a	Relative fluorescence intensity			
	$\overline{I_{N_2}}$	I _{air}	<i>I</i> ₀₂	Quenching ^b
γ-CD				
$E(\lambda = 400 \text{ nm})$	1	1	0.98	2
$M(\lambda = 360 \text{ nm})$	1	1	1	0
β-CD				
$E(\lambda = 420 \text{ nm})$	1	0.76	0.36	64
$M(\lambda = 360 \text{ nm})$	1	1	1.02	0

Effect of oxygen on the fluorescence intensity of B_5 in aqueous solutions of γ -CD and β -CD

^aE and M denote excimer and monomer respectively.

^bExpressed as a percentage:

is enhanced compared with that in aqueous solution. This observation is often taken as evidence of the formation of an inclusion complex [14]. The enhancement of B_n fluorescence, particularly the intramolecular excimer fluorescence via association with β -CD and γ -CD, indicates that B_n can form a two-to-one guest host complex with β -CD and γ -CD. Examination of molecular models suggests that a B_n molecule may be buried entirely in a γ -CD cavity, where the polymethylene chain folds together and the two naphthalene rings adopt a planar sandwich configuration. Thus, γ -CD- B_n complexes exhibit only excimer fluorescence with the exception of the γ -CD- B_2 complex which shows both monomer and excimer emission: the polymethylene chain of B_2 is too short to enable the two end naphthalene rings to attain a sandwich configuration.

Two plausible but structurally different complexes (endo and exo) which favour excimer formation are shown in Fig. 7. The whole B_n molecule in either conformation can be deeply buried in the γ -CD cavity; hence the hydrophobic environment around the probes protect it from quenching by sodium nitrite located in the aqueous phase. It has been proposed [15] that the CD cavity strongly inhibits quenching of aromatic luminophors by dissolved oxygen. The data in Table 1 show that γ -CD provides substantial protection of both excimer and monomer emission of the B_n from quenching by O_2 .



Fig. 7. Conformation of B_n -CD inclusion complex.

Inspection of molecular models indicates that the cavity of β -CD can accommodate only one naphthalene ring. Two naphthalene rings in a sandwich configuration can hardly reach the bottom of the β -CD cavity. However, an exo form of the two-to-one inclusion complex as shown in Fig. 7 is still possible, in which part of the two naphthalene moieties is buried in the β -CD cavity and the rest is exposed to water. Since the naphthalene groups are not completely buried, the excimer fluorescence is readily quenched by the sodium nitrite and the oxygen in the water (Table 1). Furthermore, compared with γ -CD-B_n complexes (excimer fluorescence, $\lambda_{max} = 400$ nm), the excimer fluorescence of β -CD-B_n shows a red shift ($\lambda_{max} = 420$ nm). This is probably due to the larger energy of repulsion between the two ground state chromophores in β -CD-B_n complexes than in γ -CD-B_n complexes [16].

An exo configuration for the β -CD-B_n complexes is required to explain the ratio of excimer to monomer emission. γ -CD-B₂, as well as β -CD-B₂, shows both monomer and excimer fluorescence ($I_E/I_M = 2.7$), again owing to the short polymethylene chain. For B₃ and B₄ complexes with β -CD, $I_E/I_M >$ 10 which implies that two-to-one complexes can be formed readily. B₅ and B₁₀, with their longer polymethylene chain, occupy more space in the β -CD cavity and leave less room for accommodating the naphthalene rings, so the formation of a two-to-one inclusion complex becomes more difficult.

Because the size of the α -CD cavity is small, it cannot even form a oneto-one complex with naphthalene. However, adding α -CD to aqueous B_4 , B_5 or B_{10} enhances the monomer fluorescence. We suggest that the polymethylene chain is folded and associates with the α -CD. Thus, the naphthalene ring is forced to enter the α -CD cavity and is partly protected from quenching. The polymethylene chains of B_2 and B_3 are too short to be folded so they cannot form stable inclusion complexes with α -CD, and adding α -CD produces no effect on the B_2 and B_3 emission.

When the concentration of β -CD and γ -CD is increased, the ratio of the excimer to monomer emission decreases. This result suggests that an association equilibrium exists between the two-to-one and one-to-one complex.



N represents the naphthalene ring. Apparently, increasing the CD concentration favours one-to-one complex formation, and hence I_E/I_M decreases.

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