Studies on the interactions of 2,5-diphenyl 1,3,4-oxadiazole and 2,5-diphenyl 1,3-oxazole with β-cyclodextrin*

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Abstract Interactions of 2,5-diphenyl 1,3,4-oxadiazole (PPD) and 2,5-diphenyl 1,3-oxazole (PPO) with β-cyclodextrin (β-CD) are studied by ^{1}H-NMR and steady-state fluorescence measurements, and the stoichiometries and the association constants are estimated. It is found that the hydrophobic interaction is the main driving force for the formation of inclusion complexes of PPD and PPO with β-CD. In the presence of aliphatic alcohols (from 1-propanol to 1-pentanol), PPD and PPO transfer from the CD cavity to the aqueous phase. Quenching experiments of PPD and PPO by iodide further prove the above conclusions. The results suggest that stereo effect is the crucial factor to the inexistence of nanotube in PPD (or PPO)-β-CD systems.

Keywords: ^{1}H-NMR, steady-state fluorescence, fluorescence anisotropy, β-cyclodextrin.

Cyclodextrins (CDs) are cyclic oligosaccharides that have the ability to selectively incorporate various inorganic or organic compounds[1,2]. Studies on "molecular necklace" type complexes have received increasing interest in recent years[3~5]. Li et al. reported that[3] β-CD or γ-CD can form nanotube by including the rodlike molecule, i.e. all-*trans*-1,6-dipheny-1,3,5-hexatriene (DPH). Agbaria and his coworker[4,5] found that some oxazole molecules, such as 2,5-diphenyl 1,3-oxazole (PPO), 2,5-diphehyl 1,3,4-oxadiazole (PPD), 2-phehyl-5-(4-diphenyl) 1,3,4-oxadiazole (PBD) and 2,5-(4,4′-diphenyl) 1,3,4-oxazole (BBOD) could form 2:1 binary inclusion complexes with γ-CD. At higher concentration, these units form extended nanotubes. The formation of these nanotubes is reversible when heating and cooling, similar to the behavior of temperature-dependent liquid crystals. It has also been found that these oxazoles cannot form nanotube with β-CD. Up to now, only five small molecules, i.e. DPH, PPD, PPO, PBD and BBOD have been reported to form nanotube with CDs. Thus, it is theoretically important to study the mechanism of the formation of the nanotube[6,7]. This may help searching for more such small molecules.

In this paper, we first aim at studying the formation of inclusion complexes between β-CD and PPD (or PPO) by means of ^{1}H-NMR measurement, and absorption and fluorescence spectral techniques. The detailed information on the interactions of PPD and PPO with β-CD does not exist in the literature. In order to explore the reason why the nanotube cannot be formed in these systems, we also want to study the interaction between aliphatic alcohols, PPD (or PPO) and β-CD. To elucidate whether the ternary inclusion complex is formed or not will help us to explain the factors affecting the formation of the nanotube.

1 Experiments

1.1 Reagents and instruments

PPD and PPO (Aldrich) were chromatographed by a silica gel column. β-CD and KI (Beijing Shuanghuan Reagent Plant, China) were recrystallized three times. D_2O (99.9%) was purchased from Cambridge Isotope Laboratory Inc., USA. All other reagents were of analytical grade.

^{1}H-NMR spectra were run on an ARX-400 spectrometer. At least 800 transients were collected for each ^{1}H-NMR spectrum. Chemical shifts were expressed relative to an added internal standard of methanol ($\delta = 3.4000 \times 10^{-6}$)[8]. Absorption and fluorescence spectra were recorded on a Shimadzu UV-3100 and a Hitachi F-4500 fluorescence spectra photometer, respectively. Fluorescence anisotropy was recorded on a Shimadzu RF-5301 photometer. Excitation wavelengths of PPD and PPO are 280 nm and 313 nm, respectively.

1.2 Methods

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Stock solutions of PPD and PPO were prepared by dissolving them in methanol. Appropriate amounts (volume fraction φ no more than 1%) of stock solutions and β-CD were added to a 5 mL flask, and diluted to the mark with triply distilled water. All measurements were carried out at room temperature.

2 Results and discussion

2.1 Spectral characteristics

2.1.1 Absorption spectra Fig. 1 shows the absorption spectra of PPD in the solutions of β-CD at different concentrations. With the increase in the β-CD concentration, the absorption intensity slightly decreases accompanied by an isosbestic point at 294 nm, indicating that PPD may form an inclusion complex with β-CD.

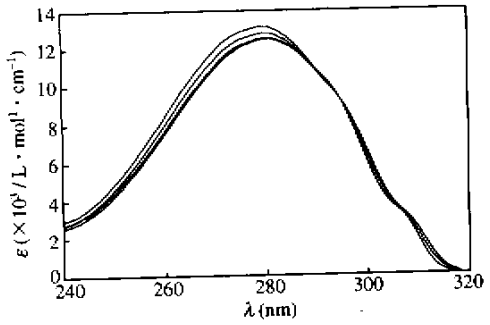

Fig. 1. UV spectra of PPD (4.0×10^{-5} mol/L) in β-CD solution. From top to bottom: [β-CD] = 0, 8×10^{-4}, 2×10^{-3}, 1×10^{-2} mol/L.

2.1.2 Fluorescence spectra Fig. 2 depicts fluorescence spectra of PPD and PPO at different β-CD concentrations. It can be seen that PPD exhibits two peaks at 333 and 346 nm, respectively, and PPO has only one distinct peak at 375 nm. The fluorescence intensities of PPD and PPO are reduced by the addition of β-CD, which should result from the fluorescence quenching mechanism. The role of the nitrogen heteroatom as a symmetry interrupting entity has been regarded as an important factor in the quenching mechanism of the molecules containing heteroatom rings[9].

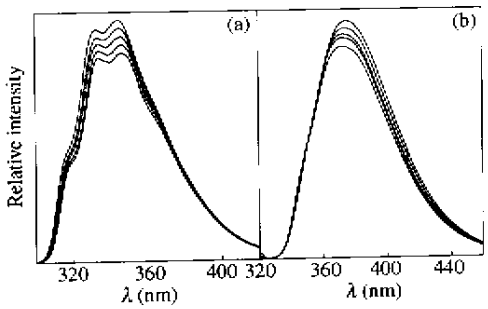

Fig. 2. Fluorescence spectra of PPD (2.4×10^{-6} mol/L) (a) and PPO (4.0×10^{-3} mol/L) (b) in β-CD solution. From top to bottom: [β-CD] = 0, 4×10^{-4}, 1×10^{-3}, 4×10^{-3}, 1×10^{-2} mol/L.

2.1.3 ^{1}H-NMR spectra The ^{1}H-NMR spectra of PPD in the aqueous solutions of β-CD in D_2O at varying concentrations are shown in Fig. 3. Three kinds of hydrogen (H_a, H_b, H_c) in PPD are also shown.

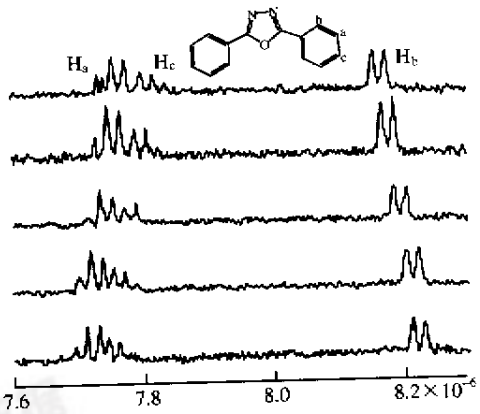

Fig. 3. ^{1}H-NMR spectra of PPD (4.0×10^{-5} mol/L) in β-CD solution. From top to bottom: [β-CD] = 0, 2×10^{-4}, 8×10^{-4}, 2×10^{-3}, 4×10^{-3} mol/L.

According to Fig. 3, upon the addition of β-CD, the signal of the proton H_b is higher-field shifted, showing the shielding effect on the H_b proton. The addition of β-CD also results in downfield shifts of H_a and H_c, and the shift value of H_c is larger than that of H_a. These indicate that attraction to the electron of C = N bond in the oxazole ring decreases, which results in increase in the charge density of C_b. Thus, one can conclude that the oxazole ring enters into the inner cavity of β-CD. As a result, the hydrogen bond, formed between N atom and H_2O molecule is broken. Although N atom might form hydrogen bond with —OH bond inside the β-CD cavity, this force is evidently weaker than that in pure water.

2.2 Stoichiometries and association constants

2.2.1 NMR titration method Taking into account the sizes of PPD and the inner cavity of β-CD, we consider the following stepwise equilibra[1]:

$$\mathrm{PPD} + \mathrm{CD} \overset{K_1}{\rightleftharpoons} \mathrm{PC}, \quad (1)$$

$$\mathrm{PC} + \mathrm{CD} \overset{K_2}{\rightleftharpoons} \mathrm{PC_2}, \quad (2)$$

where PC, PC_2 denote the 1∶1 and 1∶2 inclusion complexes, respectively, while K_1 and K_2 are the association constants. Then the chemical shift displacement can be calculated according to the following equation[1]:

$$\Delta = \frac{\Delta_{11}K_1[\mathrm{CD}] + \Delta_{12}K_1K_2[\mathrm{CD}]^2}{1 + K_1[\mathrm{CD}] + K_1K_2[\mathrm{CD}]^2}, \quad (3)$$

where Δ_{11} and Δ_{12} represent the chemical shift displacements of the 1∶1 and 1∶2 inclusion complexes to that of PPD in pure D_2O, respectively. In this study, the equilibrium concentration of cyclodextrin, [CD], can be replaced by its initial concentration, $[\mathrm{CD}]_0$, since it is much larger than the concentration of PPD.

The fit based on Eq. (3) cannot give a reasonable result. So, we consider the following two special cases.

Case 1. Only the 1∶1 complex is formed, i.e. $K_2 = 0$. Thus, Eq. (3) becomes

$$\Delta = \frac{\Delta_{11}K_1[\mathrm{CD}]_0}{1 + K_1[\mathrm{CD}]_0}. \quad (4)$$

Case 2. Only the 1∶2 complex is formed. Then

$$\Delta = \frac{\Delta_{12}K_1K_2[\mathrm{CD}]_0^2}{1 + K_1K_2[\mathrm{CD}]_0^2}. \quad (5)$$

The NLR analyses of the Δ values of H_a and H_b with [CD] were carried out on the basis of Eqs. (4) and (5). Only the fit based on Eq. (4) is reasonable (see Fig. 4), which indicates that only the 1∶1 complex is formed. The parameter K_1 is estimated at (672 ± 30) and (609 ± 25) $\mathrm{mol^{-1}L}$ for H_a and H_b, respectively.

The Benesi-Hildebrand double reciprocal plot was also obtained. Only $1/\Delta$ versus $1/[\mathrm{CD}]$ exhibits a straight line (figure not shown). This further supports the 1∶1 model.

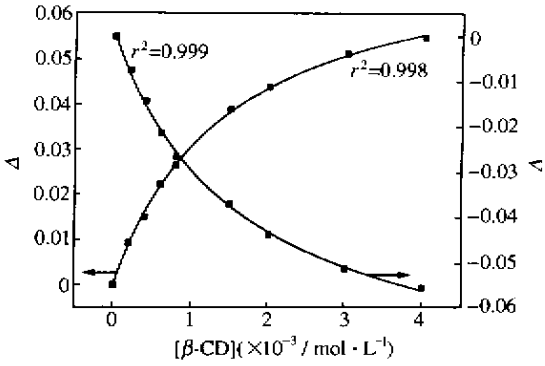

Fig. 4. Plots of the chemical shift (■ H_a, ● H_b) versus [β-CD] for PPD.

2.2.2 Fluorescence anisotropy methods Fig. 2 shows that the addition of β-CD has little effect on the fluorescence intensity of PPD and PPO, so it is not suitable to calculate the association constants by fluorescence intensity method. As we know, the dependence of fluorescence anisotropy R upon rotational diffusion has been used to quantify protein-ligand association constant[10], while this method is seldom used to estimate the association constants of fluorophore-CDs[11]. Our results show that the anisotropy of PPD (or PPO) was fairly influenced by the addition of β-CD, so it is possible to estimate the association constants from fluorescence anisotropy measurements.

Assume that fluorophore can form a 1∶1 inclusion complex with β-CD. The fluorescence anisotropy R could be related to $[\mathrm{CD}]_0$ by the following equation[11]:

$$[\mathrm{CD}]_0 = \frac{R - R_F}{K_1(R_B - R)}. \quad (6)$$

In the PPD-β-CD solution, it was found that the NLR analysis based on Eq. (6) gave reasonable result. K_1 was estimated at (562 ± 43) $\mathrm{mol^{-1}L}$, with a correlation coefficient $r^2 = 0.99$ (Fig. 5). The value of K_1 is in good agreement with the NMR titration method, which suggests the method of fluorescence anisotropy is reasonable. It was aslo found that PPO and β-CD could form the

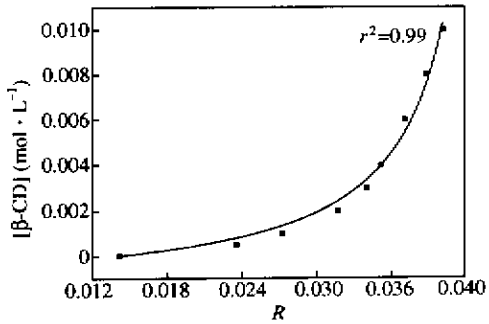

Fig. 5. Plot of [β-CD] versus R for PPD.

1∶1 inclusion complex, and $K_1 = (408 \pm 37)\ mol^{-1}L$ ($r^2 = 0.99$, figure not shown).

It has been reported that when the interactions of two guests with β-CD are hydrophobic in nature, the ratio of $\ln[K_1/(mol^{-1}L)]$ is equal to that of the molecular volume[1]. In this experiment, the ratio of $\ln K_1$ for PPD-β-CD and PPO-β-CD systems is 1.06 ± 0.03. This value is close to 1, while the volumes of PPD and PPO are nearly the same. This suggests that the interactions between these two guest molecules and β-CD are mainly hydrophobic. In fact, the hydrogen bonding does exist in the aqueous solution of oxazoles. Troler found that[12] PPD with two N atoms only formed one hydrogen bond in pure water. Therefore, it is safe to say that both PPD and PPO can only form one hydrogen bond with —OH bond in the β-CD cavity. As a result, the difference of the association constants of these two systems is decided by the little difference of their hydrophobility.

2.3 Ternary inclusion complexes

A series of fluorescence spectra were recorded for PPD (and PPO) in aqueous solution containing 0.1 mol/L 1-propanol or 1-pentanol, at various concentrations of β-CD. The results showed that fluorescence spectra were nearly the same with or without alcohol at the same β-CD concentration, except that the fluorescence intensity was higher in 1-pentanol system than that in other alcohol systems. This suggests that alcohols only react with β-CD. Because 1-pentanol interacts more strongly with β-CD molecules, the amount of the oxazole molecule included by β-CD is less in this system and its fluorescence is quenched more slightly. The fit based on the model of 1∶1∶1 ternary inclusion complex[1] could not give a reasonable result.

The ^{1}H-NMR spectra have been obtained for the systems composed of β-CD, PPD, and 0.1 mol/L propanol or butanol (results not shown). The chemical shift value of PPD is not changed in the presence of alcohols, indicating that alcohols do not react with PPD. Comparing the ^{1}H-NMR spectra of PPD in the solution with or without alcohols at the same β-CD concentration, one found no distinct difference, except that the chemical shift displacement of PPD is lower when adding alcohols. This may suggest that the alcohols are included in the β-CD cavity, so that PPD is excluded into the bulk.

One can fit the data of the chemical shift of PPD in the ternary system according to Eq. (4) by replacing $[CD]_0$ with $[CD]_e$, which is the equilibrium concentration of β-CD after reacting with alcohols, and reasonable results can be obtained with a correlation coefficient of $r^2 = 0.99$. Association constants K_1 are (598 ± 44) and $(602 \pm 22)\ mol^{-1}L$ for 1-propanol and 1-butanol system respectively. These values are very close to those in the binary systems. Thus, the possibility of the formation of ternary complex can be totally ruled out.

2.4 Studies on quenching of PPD and PPO fluorescence

To further investigate the influence of alcohols on β-CD-PPD (or PPO) inclusion complexes, a series of fluorescence quenching experiments were carried out by using KI as a quencher. In each system, linear Stern-Volmer plot can be obtained, and the Stern-Volmer constants are presented in Table 1.

Table 1. Stern-Volmer constants ($K_{SV}/mol^{-1}L$) of PPD and PPO systems

Medium	K_{SV} of PPD	K_{SV} of PPO
Water	32.5 ± 1.0	21.9 ± 0.9
5 mmol/L β-CD	22.8 ± 0.6	12.0 ± 0.6
5 mmol/L β-CD + φ1% 1-propanol	23.2 ± 0.8	13.1 ± 0.7
5 mmol/L β-CD + φ1% 1-pentanol	26.6 ± 1.2	14.0 ± 0.9

Table 1 shows that upon adding β-CD to PPD or PPO solution the Stern-Volmer constant decreases, indicating that when guest molecules and β-CD form the inclusion complex, their excited states are protected to a certain extent. It can be inferred that I^- does not enter into the β-CD cavity, otherwise the guest molecules are more easily quenched in the cavity owing to the formation of exciplex[13]. The value of Stern-Volmer constant is slightly larger with alcohols than that without them, which leads to the conclusion that alcohols react with β-CD, and then exclude PPD (or PPO) into the bulk from β-CD cavity.

2.5 Discussion

Having ascertained the mechanism of the interaction between PPD (or PPO) and β-CD, we can now discuss the structural feature of the 1∶1 binary inclusion complexes between these guest molecules and β-CD.

Agbaria and his coworker[4,5] assumed that the heterocyclic middle ring of PPO is hydrophilic, so that only one phenyl ring of PPO enters the CD cavity. On this assumption, they proposed the feasible structure of PPO-γ-CD nanotube. At lower concentration, two PPO molecules could partly enter the cavity of one γ-CD from two sides to form the 2∶1 binary inclusion complex (the notation is PCP). When the concentration of PCP increases, these units could be linked by overlapping pairs of PPO molecules to form nanotube (namely PCPPCPP……CP). In view of the narrow cavity of β-CD, they suggested that

it was impossible for β-CD to form the 2∶1 binary inclusion complex and the nanotube with PPO. Moreover, they inferred that there is not a 1∶1 binary complex (PC) in PPO-β-CD system, or else at higher concentration, these units would aggregate to form nanotube linked by single PPO (PCPCPC……).

However, our results strongly suggest that both the phenyl ring and part of oxazole ring can enter the β-CD cavity, which prevents the occupied cavity from including another PPO to form the 1∶2 binary inclusion complexes, and it is impossible to form the nanotube. The above results indicate that stereo selectivity is a crucial factor to the inexistence of nanotube between PPO (PPD)-β-CD systems. The nonentity of the 1∶1∶1 ternary inclusion complex further confirms that guest molecules occupy most part of the β-CD cavity, so that there is no more room for alcohols to enter.

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