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Effects of Cyclodextrins on the Fluorescence of Diphenyl Phosphate in Aqueous Solution

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The effects of α-, β-, and γ-cyclodextrin (CD) on intramolecular excimer formation between the two phenyl groups of diphenyl phosphate (DP) in aqueous solution were investigated by means of absorption and fluorescence spectra. It was found that DP forms 1 : 1 inclusion compounds with α-, β-, and γ-CDs. In the inclusion compound with α- or β-CD, only one phenyl group of a DP molecule is incorporated in the cavity of a CD molecule. In the case of γ-CD, however, the cavity can accommodate either one or two phenyl groups of a DP molecule. The pH dependence of the equilibrium constant for the formation of the inclusion compound between DP and β-CD has also been examined. Over the pH range from 3.46 to ≈10 the equilibrium constant does not change, whereas for pH values above ≈11 it decreases drastically.

Cyclodextrins (CDs) are cyclic oligosacharides consisting of more than five glucopyranose units. CDs containing 6, 7, and 8 glucose units are specified as α-CD, β-CD, and γ-CD, respectively. The effects of these CDs on the fluorescence properties of various aromatic compounds have been studied extensively.[1–8)] The fluorescence intensity of a guest molecule is usually enhanced by the formation of an inclusion compound with CD.[1–5,7,8)]

There have been several studies on the inclusion compounds of CDs with 1,3-bichromophoric substances which form an intramolecular excimer between two biphenyl groups or two naphthyl groups.[9–12)] Bis(4-biphenylylmethyl)ammonium chloride (BBA) exhibits both a monomer and an excimer fluorescence in aqueous solution. The effects of CDs on the two emission bands have been reported by Emert et al.[9)] The addition of α-CD to a BBA aqueous solution results in the reduction of the excimer fluorescence. No excimer peak is observed when β-CD is added. In contrast, excimer fluorescence is enhanced by the addition of γ-CD. The authors have suggested that the smaller cavity of α- and β-CDs which can accommodate only one aryl residue inhibits excimer formation, whereas the larger cavity of γ-CD, which can host two aryl moieties, enhances excimer formation considerably. For 1,3-bichromophoric substances possessing two naphthyl groups, it has also been shown that two naphthyl groups in the same molecule can be located inside the γ-CD cavity.[9–12)]

Among the 1,3-bichromophoric compounds investigated up to now, dibenzylammonium chloride (DA) is the only compound that possesses two phenyl groups.[9)] The addition of α-, β-, and γ-CDs have no effects on both the monomer and the excimer fluorescence of DA. This result has been explained as due to the prevention of a deep penetration of DA by both the second benzene moiety and the positively charged nitrogen.

Although the formation of an inclusion compound with CD is affected by other factors than the cavity size of CD and the molecular size and shape of a guest molecule, it appears that two phenyl groups, which are less bulky than two naphthyl groups, can be incorporated in the same γ-CD cavity. Thus, as a 1,3-bichromophoric compound having two phenyl groups, we selected diphenyl phosphate which was found to show both monomer and excimer fluorescence in aqueous solutions, and investigated the formation of inclusion compounds of diphenyl phosphate with α-, β-, and γ-CDs by means of absorption and fluorescence spectra.

Experimental

Diphenyl phosphate, purchased from Nakarai, was recrystallized twice from cyclohexane. β-Cyclodextrin (β-CD) from Nakarai was recrystallized three times from water. α- and γ-CDs obtained from Nakarai and Tokyo Kasei, respectively, were used without further purification.

The absorption spectra were measured with a Shimadzu 260 spectrophotometer, and the fluorescence spectra were recorded on a Shimadzu RF-501 spectrofluorometer equipped with a cooled EMI 9789QA photomultiplier. Fluorescence was viewed at right angles to the direction of excitation, and the spectra were corrected for the spectral response of the detection system as previously described.[5)] The absorption and fluorescence spectra were measured at 25±0.1 °C unless otherwise stated. Aerated sample solutions were used throughout this work.

Results and Discussion

Complex Formation of Diphenyl Phosphate (DP) with α- and β-Cyclodextrins (CDs). Since DP is a monoprotic acid, DP can take both anionic and neutral forms. By using a conventional titration method, the $\mathrm{p}K_\mathrm{a}$ of DP was determined to be 2.4. At the DP concentration (5×10^{-4} mol dm^{-3}) used for this work, the pH of an aqueous DP solution was 3.46. Therefore, the DP at 5×10^{-4} mol dm^{-3} is predominantly in its anionic form in aqueous solution.

Figure 1 shows the absorption spectra of 5×10^{-4} mol dm^{-3} DP in aqueous solutions containing varying concentrations of β-CD. The absorption band of DP is red-shifted with isosbestic points at 228 and 254 nm as the concentration of β-CD is increased. This finding indicates a simple equilibrium involving a 1:1

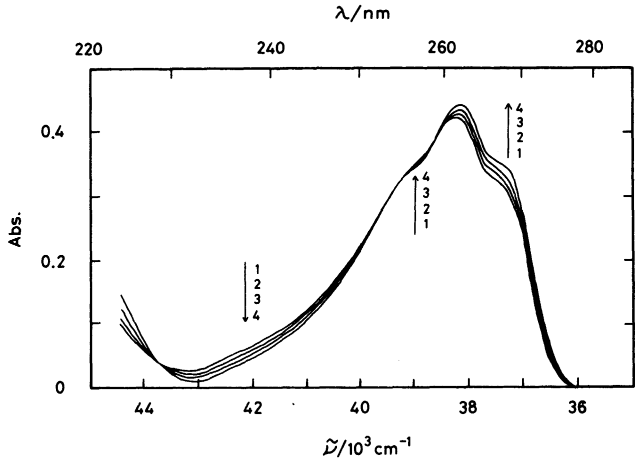


Fig. 1. Absorption spectra of DP (5×10^{-4} mol dm^{-3}) in aqueous solution in the presence of β-CD. Concentration of β-CD: (1) 0 mol dm^{-3}; (2) 10^{-3} mol dm^{-3}; (3) 2×10^{-3} mol dm^{-3}; (4) 5×10^{-3} mol dm^{-3}; (5) 10^{-2} mol dm^{-3}.

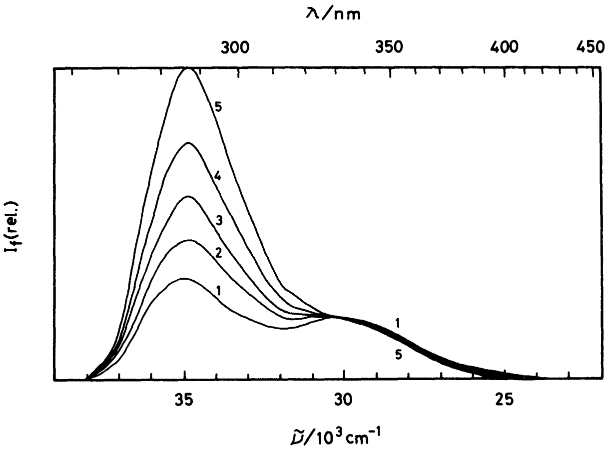
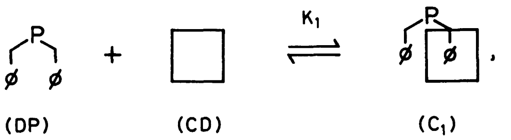


Fig. 2. Fluorescence spectra of DP (5×10^{-4} mol dm^{-3}) in aqueous solution in the presence of β-CD. Concentration of β-CD: (1) 0 mol dm^{-3}; (2) 10^{-3} mol dm^{-3}; (3) 2×10^{-3} mol dm^{-3}; (4) 4×10^{-3} mol dm^{-3}; (5) 10^{-2} mol dm^{-3}.

inclusion compound of DP formed with β-CD. The equilibrium between DP and a 1:1 inclusion compound of DP with β-CD can be represented by the following scheme:



(1)

where C_1 is a 1:1 inclusion compound of DP with CD in which only one phenyl group of a DP molecule is incorporated in the cavity of a CD molecule, and K_1 is an equilibrium constant for the formation of C_1. Because of a very small variation in the absorbance, we could not reliably determine the value of the equilibrium constant from the absorption data.

Figure 2 illustrates the fluorescence spectra of DP (5×10^{-4} mol dm^{-3}) in aqueous solutions with varying concentrations of β-CD. The fluorescence spectrum of DP alone exhibits two bands whose maxima are at 286 and 330 nm, respectively. The 286-nm band can be assigned to a monomer fluorescence since phenyl dihydrogenphosphate shows only one fluorescence band with a maximum at 288 nm in aqueous solution. The 330-nm band can be assigned to the intramolecular excimer fluorescence of DP from the following. (1) Even at a DP concentration as low as 5×10^{-5} mol dm^{-3}, the fluorescence spectrum was identical to that for a 5×10^{-4} mol dm^{-3} DP solution. (2) The excitation spectrum for the 330-nm band was the same as that for the 286-nm band. (3) The maxima of the excimer emissions of benzene and 1,3-diphenylpropane are reported to be 320 and 333 nm, respectively.[13,14] Corresponding to the C–C–C carbon-atom skeleton in the cases of 1,3-bichromophoric propanes, the three-atom molecular chain, O–P–O, in a DP molecule is responsible for the formation of the intramolecular excimer.

The addition of β-CD results in a remarkable enhancement of the DP monomer band without a shift in the maximum position of the monomer band. In contrast to the dibenzylammonium chloride studied by Emert et al., the effect of β-CD on the DP fluorescence is evident. To evaluate the intensity of excimer emission, the fluorescence spectrum of phenyl dihydrogenphosphate was assumed to be the same as that of the DP monomer, and the tail of its emission, which extends to ≈380 nm, was subtracted from the excimer portion of the spectrum. The true intensity of the excimer band decreased upon increasing the β-CD concentration.

In the cases of benzene and naphthalene derivatives, the equilibrium constants for the formation of inclusion compounds, obtained from the absorption and fluorescence spectrophotometric methods, almost coincide with each other.[5,16] The rate constants of the association and dissociation reactions for the systems of a naphthalene derivative and β-CD are of the order of 10^7 mol^{-1} s^{-1} and 10^4 s^{-1}, respectively.[15] Although the corresponding rate constants for DP are not available, the above values are small compared with the expected decay rate constant (the order of 10^8 s^{-1}) of the singlet excited state of DP. Therefore, it is likely that an equilibrium between excited DP and β-CD cannot be established within the lifetime of excited DP. Thus, K_1 was evaluated to be 200 mol^{-1} dm^3 from the intensity change in the monomer fluorescence of DP, according to the method previously described.[5] This value is in good agreement with the equilibrium constants reported for the 1:1 inclusion compound formed between benzene and β-CD (196±10 (28 °C) and 169±1 (25 °C) mol^{-1} dm^3).[16,17]

When α-CD was added to a DP aqueous solution, similar changes were observed both in the absorption

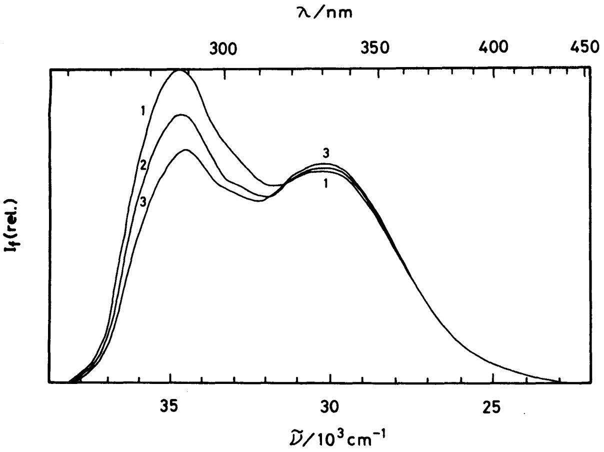


Fig. 3. Fluorescence spectra of an aqueous DP (5×10^{-4} mol dm^{-3}) solution in the presence of γ-CD at 43 °C. Concentration of γ-CD: (1) 0 mol dm^{-3}; (2) 10^{-2} mol dm^{-3}; (3) 2×10^{-2} mol dm^{-3}.

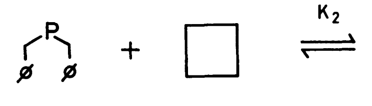
and fluorescence of DP except that these changes were much smaller than those for β-CD, indicating the formation of the C_1 inclusion compound between DP and α-CD to a smaller extent. For α-CD, K_1 was determined to be 0.85 mol^{-1} dm^3 from measurements of the DP monomer fluorescence. The equilibrium constant for the formation of the 1:1 inclusion compound between benzene and α-CD has been reported to be 31.6±0.1 mol^{-1} dm^3 at 25 °C.[17] A marked disparity between the equilibrium constant of α-CD with DP and that with benzene suggests a strong obstruction against complex formation by the substituent group (-O-P(-O$^-$)(=O)-O-Ph) in the DP molecule.

Table 1. ΔH and ΔS for the Formation of the 1:1 Inclusion Compounds (C_1) of DP with α- and β-CDs

	DP		Benzene$^{a)}$	
	α-CD	β-CD	α-CD	β-CD
ΔH/kJ mol^{-1}	−37	−8.0	−13.1	−1.9
ΔS/J K^{-1} mol^{-1}	130	15	−15.5$^{b)}$	36.1$^{b)}$

a) From Ref. 17. b) Calculated from the equilibrium constant and the corresponding enthalpy change shown in Table 1 of Ref. 17.

Complex Formation of DP with γ-CD. Below a γ-CD concentration of ≈3×10^{-2} mol dm^{-3}, the spectral change in the DP absorption was similar to that for β-CD. However, above a γ-CD concentration of ≈3×10^{-2} mol dm^{-3}, no isosbestic points were observed in the absorption spectra. This finding indicates the coexistence of at least two different equilibria between DP and γ-CD at a high γ-CD concentration. In this work, therefore, we restricted the γ-CD concentration up to 3×10^{-2} mol dm^{-3}. We found that a spectral change of the DP fluorescence caused by the addition of γ-CD varied significantly with temperature. In the lower-temperature range (below ≈10 °C), the DP monomer fluorescence was enhanced with a decrease in the excimer fluorescence by the addition of γ-CD. As in the cases of α- and β-CDs, the enhancement of the monomer fluorescence is due to the formation of the 1:1 inclusion compound, C_1, of DP with γ-CD. From the fluorescence measurements, K_1 was evaluated to be 1.5 mol^{-1} dm^3 at 3 °C. Figure 3 shows the DP fluorescence spectra at 43 °C in the absence and presence of γ-CD. A decrease in the DP monomer fluorescence was observed in the higher-temperature range (above ≈25 °C), accompanied by an enhancement of the DP excimer fluorescence. The result in the higher-temperature range is similar to that for 1,3-bichromophoric compounds possessing two naphthyl or two biphenyl groups, indicating that two phenyl groups in a DP molecule are inserted into the same cavity of a γ-CD molecule.[9–12] The change in the DP fluorescence spectra in the higher-temperature range can be interpreted by the following equilibrium:



(2)

where C_2 is an inclusion compound in which two phenyl groups in a DP molecule are incorporated in the same cavity and K_2 is an equilibrium constant for the formation of C_2. In the case of C_2, it is likely that excited DP changes its geometrical conformation inside the γ-CD cavity within its lifetime and emits both a monomer and an excimer fluorescence. Even at a high temperature, C_1 in Eq. 1 can exist in a DP solution containing γ-CD. Since C_1, if it exists, contributes to both an increase in the monomer band and a decrease in the excimer band, the reverse intensity changes, a decrease in the monomer band and an increase in the excimer band (Fig. 3), is due mainly to the existence of C_2. By assuming that the presence of C_1 was negligible, K_2 at 43 °C was determined to be 30 mol^{-1} dm^3 from the intensity change in the monomer fluorescence. At such a high temperature as 43 °C, the increase in the DP excimer fluorescence at the expense of the DP monomer fluorescence may suggest that the enthalpy change (ΔH) for the C_2 formation is close to zero and, on the other hand, the entropy change (ΔS) for that is a positive value. Unfortunately, the thermodynamic parameters for K_1 and K_2 in the DP–γ-CD system could not be estimated because neither C_1 nor C_2 could be ignored over the fairly wide temperature range examined.

Temperature Dependence of K_1 for α- and β-CDs. From the temperature dependence of K_1 for α- and β-CDs, ΔH, and ΔS for the formation of C_1 could be obtained. The results are listed in Table 1 together with the data of benzene reported by Tucker and Christian.[16] For the inclusion compound of β-CD, positive values of

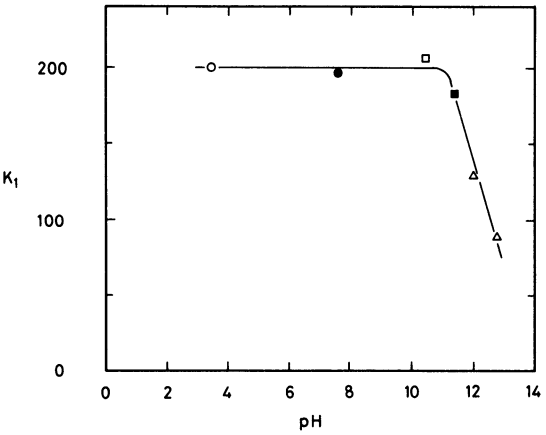


Fig. 4. pH dependence of K_1 for β-CD: (○) without buffer; (●) KH_2PO_4–NaOH buffer; (□) $NaHCO_3$–NaOH buffer; (■) Na_2HPO_4–NaOH buffer; (△) KCl–NaOH buffer.

ΔS were obtained for both DP and benzene. The larger $-\Delta H$ value for DP than that for benzene suggests that the substituted benzene ring fits more tightly the size of the β-CD cavity than the unsubstituted benzene nucleus. In the case of α-CD, ΔS for benzene is negative, whereas that for DP is positive. This result may show that a benzene molecule is more rigidly incorporated in the α-CD cavity than a DP molecule is. The larger $-\Delta H$ of α-CD both for DP and benzene than those of β-CD reveal that the cavity size of α-CD is more appropriate to the complexation with the benzene ring than that of β-CD.

pH Dependence of K_1 for β-CD. In order to further study the inclusion processes of DP, we examined the pH dependence of K_1 for β-CD. In Fig. 4, the values of K_1 are plotted as a function of pH. K_1 is constant at pH values between 3.46 and $\approx$10. However, K_1 abruptly decreases as the pH is increased above $\approx$11. The pK_a value for α-CD is known to be 11.8.[18] Since both α- and β-CDs are constituted from the identical glucopyranose units, nealy the same value of pK_a is expected for β-CD. As already mentioned, DP exists as an anionic form over the whole pH range studied. Therefore, the sharp drop of K_1 in Fig. 4 indicates an electrostatic repulsion between an anionic DP and an anionic β-CD molecule. In addition, if the deprotonation of β-CD at pH$\approx$11 causes a conformation change in β-CD, this change can play an important role in the reduction of K_1.

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