Fluorescence and Induced Circular Dichroism Studies on Host–Guest Complexation between γ-Cyclodextrin and Pyrene

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Host–guest complexation between γ-cyclodextrin (γ-CD, cyclo-octa-amylose) and pyrene has been studied in detail by fluorescene and induced circular dichroism (i.c.d.) spectra. γ-CD forms complexes with pyrene with 1 : 1, 1 : 2, 2 : 1, and 2 : 2 stoicheiometries at ambient temperature. The inclusion of pyrene by γ-CD proceeds stepwise; γ-CD including only one pyrene molecule can accommodate a second pyrene molecule to produce a 1 : 2 and/or a 2 : 2 complex. Both in a monomolecularly dispersed pyrene solution and in a suspended pyrene solution, several hours are required for γ-CD to include two pyrene molecules. The configuration of two pyrenes included is an *S*-helix. At temperatures above *ca.* 85 °C, a 1 : 1 complex alone is formed mainly, where a pyrene molecule is accommodated with its long axis parallel to the axis of γ-CD. The data are compared with those of the systems containing β-CD (cyclohepta-amylose) in place of γ-CD.

Cycloamyloses (cyclodextrins, CDs) are homologous series of non-reducing, cyclic D-(+)-glucose polymers ranging in size from six to 12 glycosyl ring structures,[1-3] and which are produced by the action of *Bacillus macerans* amylase on starch.[1,4] The number of glucose units is designated by a Greek letter: α for six, β for seven, γ for eight, and so on. The smaller α- and β-CDs have been widely utilized for various purposes,[5,6] while the use of γ- or larger CDs is still infrequent.[7-11] In recent reports of fluorescence studies,[7,8,10,11] it was suggested that γ-CD can include two molecules of benzene or naphthalene derivatives in marked contrast to α- and β-CDs which form host–guest complexes with 1 : 1 stoicheiometry. However, the details of one host–two guest complexation have remained uninvestigated. For instance, the following questions arise: (1) whether two arene molecules are included coincidentally or not, (2) what are the configurations of the arene molecules in γ-CD, and (3) how changes in temperature affect the complexation?

The present study aims to give some answers to these questions. Pyrene was selected as a representative arene. Complexation was examined by fluorescence and induced circular dichroism (i.c.d.) spectra, since the former is sensitive to the change in the polarity of the probe's environment and the latter is effective in monitoring interactions between chiral molecules and achiral chromophore molecules.[12] Also, the results are compared with those of a previous fluorescence study [13] on systems containing β-CD. Quite recently, a short communication by Kano *et al.*[14] appeared on pyrene fluorescence in the presence of γ-CD. We will cite their data as required and discuss it in comparison with our own.

Experimental

Materials.—γ-CD was a gift from Dr. N. Nakamura (Japan Maize Products Co. Ltd.). β-CD was recrystallized from distilled water. Pyrene was an analytical grade reagent available commercially and was recrystallized from ethanol.

Measurements.—Fluorescence spectra were recorded on a Shimadzu RF-500 spectrofluorophotometer, absorption spectra on a Shimadzu UV-360 spectrophotometer, and i.c.d. spectra on a JASCO J-400X spectropolarimeter. For other details, see the Figure captions.

Sample Preparation.—A fine powder of pyrene was stirred for several hours in distilled water and filtered through a G4 glass filter. Because of the low solubility of pyrene in water, the following procedure was adopted in order to estimate the concentration of pyrene in the filtrate. To a part of the filtrate was added 10^{-2}M-β-CD, the solution was aged for 24 h, and its monomer fluorescence intensity was compared with that of a standard solution containing 10^{-5}M-pyrene and 10^{-2}M-β-CD which had also been aged for 24 h. (We utilized the fact that pyrene and β-CD form an inclusion complex mainly with 1 : 1 stoicheiometry.[13] The [pyrene] in Figures 1 and 2 was estimated in this way.) A concentrated solution of pyrene in tetrahydrofuran was poured into distilled water, to which CDs were added to the desired concentrations with stirring (Figures 3—6). The volumetric ratio of tetrahydrofuran to water was always <0.0007.

Results and Discussion

Figure 1 A illustrates uncorrected fluorescence spectra of 5×10^{-7}M-pyrene in water at various γ-CD concentrations after ageing for >24 h. All the spectra contain a region from 360 to 420 nm, and some of them contain a further, broad spectral region (420—600 nm). The spectra in the former region are attributable to the monomer fluorescence of pyrene, and those in the latter to its excited dimers of excimers.[13-15] The solution was a monomolecularly dispersed pyrene solution in the absence of γ-CD (curve a).† Addition of a little γ-CD to this solution led to an increase of intensity only in monomer fluorescence region (curve b). This result suggests that only one pyrene molecule is included at this γ-CD concentration. With an increase in [γ-CD], excimer fluorescence appeared (curve c), and a further increase of [γ-CD] resulted in an increase of its intensity associated with a decrease in monomer fluorescence intensity (curves d and e). Thus these spectral changes suggest that 1 : 2 and/or 2 : 2 γ-CD-pyrene complex formation occurs after 1 : 1 complex formation. However, when [γ-CD] exceeded *ca.* 10^{-2}M, the monomer fluorescence increased again with a concomitant decrease in the excimer fluorescence intensity (not shown, see Figure 2 for details).

The intensity ratios of excimer to monomer fluorescence (E/M ratio; ratio of emission at 472 and 392 nm) of the solutions for Figure 1 A are plotted as a function of log[γ-CD] in Figure 1B. The data indicate that the ratio of pyrene excimer is larger for an old solution than for a freshly prepared solution at [γ-CD] 10^{-3}M. When β-CD was used instead, only monomer emission was observed and the intensity of the band in the old solution was larger than that of the freshly prepared one.[13]

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† The reported solubility of pyrene in water is 1.6×10^{-6}M.[16]

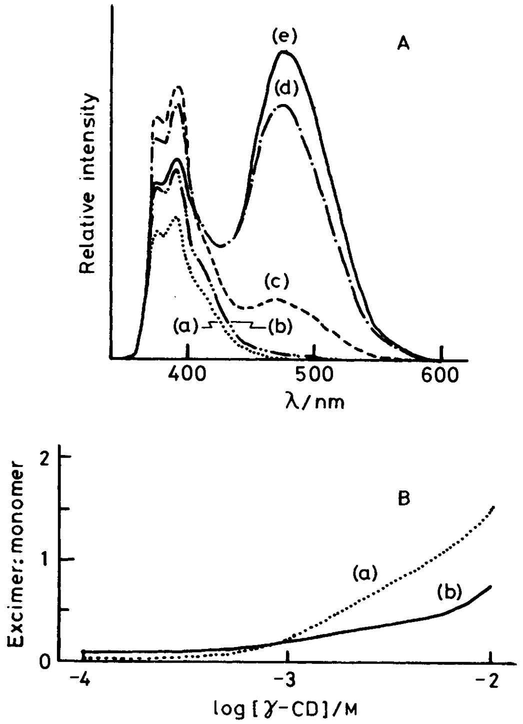

Figure 1. A, Uncorrected fluorescence spectra of pyrene (5×10^{-7}M) in water at various γ-CD concentrations: (a) 0, (b) 5.0×10^{-4}M, (c) 1.0×10^{-3}M, (d) 5.0×10^{-3}M, and (e) 1.0×10^{-2}M. Excitation was at 331 nm. Spectra were recorded after ageing for >24 h. B, Changes in the excimer : monomer ratio of pyrene (5×10^{-7}M) in water with increasing concentrations of γ-CD: (a) matured and (b) freshly prepared

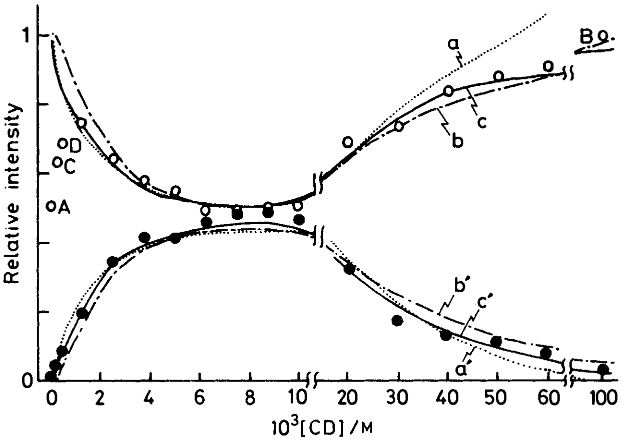

Figure 2. Changes in the monomer (○) and excimer fluorescence (●) intensities of pyrene (4×10^{-7}M) in water with increasing concentrations of γ-CD. Excitation was at 331 nm. The curves a, a′, b, b′, c, and c′ illustrate the calculated fluorescence intensities of pyrene as a function of [γ-CD] when K_1—K_4 values are the following (unit of K are l mol^{-1}): a and a′, 14, 5.3×10^7, 290, 0; b and b′, 32, 0, 570, 2.9×10^7; and c and c′, 35, 1.9×10^7, 310, 1.1×10^8. The monomer fluorescence intensity at point B was set at unity

Figure 2 shows the [γ-CD] dependence for monomer and excimer fluorescence of aged, 4×10^{-7}M-pyrene aqueous solutions. The intensities of the monomer fluorescence increased with increasing [γ-CD] to [γ-CD] *ca.* 10^{-3}M, and then decreased and again increased at [γ-CD] > *ca.* 8×10^{-3}M. The excimer emission increased to [γ-CD] *ca.* 8×10^{-3}M and then decreased in the higher [γ-CD] region. In order to interpret the effect of γ-CD on the pyrene fluorescence, equilibria (1)—(5) were assumed. However the possibility of equation (5) seemed small from the observations in Figure 1A. Thus a computer simulation was carried out for the plots in Figure 2 to estimate the K_1—K_4 values by calculating the concentration of uncomplexed pyrene, [Py], from equation (10) for given K values, [Py·γ-CD] from equation (6), [Py·Py·γ-CD] from equation (7), and [γ-CD·Py·Py·γ-CD] from equation (9). Here we assumed (i) that the uncomplexed γ-CD concentration, [γ-CD], nearly equalled the initial γ-CD concentration, [γ-CD]$_0$, because the concentration of pyrene was much lower than [γ-CD]$_0$, and (ii) that the quantum yield of excimer fluorescence of Py·Py·γ-CD complex was equal to that of γ-CD·Py·Py·γ-CD complex for simplicity. Also, the quantum yields of monomer fluorescence of Py·γ-CD and γ-CD·Py·γ-CD were supposed to be the same. Moreover we assumed the contribution of uncomplexed pyrene to the monomer fluorescence was the half that of Py·γ-CD or γ-CD·Py·γ-CD complex by comparing the monomer fluorescence intensity in the absence of γ-CD with that in the presence of large excess of γ-CD ([γ-CD]/[Py] 2.5×10^5), *i.e.*, the values at points A and B in Figure 2. In addition, the quantum yields of excimer fluorescence of Py·Py·γ-CD and/or γ-CD·Py·Py·γ-CD were supposed to be *ca.* 1.5 times larger than those of the monomer fluorescence of Py·γ-CD and/or γ-CD·Py·γ-CD, based on the observation in Figures 5 and 6. In the calculation, the fluorescence strength at point B was set at unity. We considered three cases. First, when equations (1)—(3) were taken into account (case I), well fitted curves were obtained by assuming K_1 14, K_2 5.3×10^7, and K_3 290 l mol^{-1} (curves a and a′). Secondly, equation (4) was introduced in place of equation (2) (case II), and then curves b and b′ were obtained for K_1 32, K_3 570, and K_4 2.9×10^7 l mol^{-1}. Lastly, if equations (1)—(4) were all considered (case III), curves c and c′ were best obtained at K_1 35, K_2 1.9×10^7, K_3 310, and K_4 1.1×10^8 l mol^{-1}. If K_4 was eliminated, the calculated curves deviated from the experimental points at [γ-CD] > 30mM, especially in the monomer fluorescence (curve a). And if K_2 was not introduced, errors were large for [γ-CD] < 4mM (curves b and b′). Though not shown, in the cases where both

$$\mathrm{Py} + \gamma\text{-CD} \rightleftharpoons [\mathrm{Py}\cdot\gamma\text{-CD}] \tag{1}$$

$$\mathrm{Py}\cdot\gamma\text{-CD} + \mathrm{Py} \rightleftharpoons [\mathrm{Py}\cdot\mathrm{Py}\cdot\gamma\text{-CD}] \tag{2}$$

$$\mathrm{Py}\cdot\gamma\text{-CD} + \gamma\text{-CD} \rightleftharpoons [\gamma\text{-CD}\cdot\mathrm{Py}\cdot\gamma\text{-CD}] \tag{3}$$

$$\mathrm{Py}\cdot\gamma\text{-CD} + \mathrm{Py}\cdot\gamma\text{-CD} \rightleftharpoons [\gamma\text{-CD}\cdot\mathrm{Py}\cdot\mathrm{Py}\cdot\gamma\text{-CD}] \tag{4}$$

$$\mathrm{Py}\cdot\mathrm{Py} + \gamma\text{-CD} \rightleftharpoons [\mathrm{Py}\cdot\mathrm{Py}\cdot\gamma\text{-CD}] \tag{5}$$

$$K_1 = \frac{[\mathrm{Py}\cdot\gamma\text{-CD}]}{[\mathrm{Py}][\gamma\text{-CD}]} \tag{6}$$

$$K_2 = \frac{[\mathrm{Py}\cdot\mathrm{Py}\cdot\gamma\text{-CD}]}{[\mathrm{Py}\cdot\gamma\text{-CD}][\mathrm{Py}]} \tag{7}$$

$$K_3 = \frac{[\gamma\text{-CD}\cdot\mathrm{Py}\cdot\gamma\text{-CD}]}{[\mathrm{Py}\cdot\gamma\text{-CD}][\gamma\text{-CD}]} \tag{8}$$

$$K_4 = \frac{[\gamma\text{-CD}\cdot\mathrm{Py}\cdot\mathrm{Py}\cdot\gamma\text{-CD}]}{[\mathrm{Py}\cdot\gamma\text{-CD}][\mathrm{Py}\cdot\gamma\text{-CD}]} \tag{9}$$

$$2K_1[\gamma\text{-CD}](K_2 + K_1K_4[\gamma\text{-CD}])[\mathrm{Py}]^2 + (1 + K_1[\gamma\text{-CD}] + K_1K_3[\gamma\text{-CD}]^2)[\mathrm{Py}] - [\mathrm{Py}]_0 = 0 \tag{10}$$

Calculated concentrations of uncomplexed pyrene, [Py], and γ-CD–pyrene complexes at K_1 35, K_2 1.9×10^7, K_3 310, and K_4 1.1×10^8 l mol^{-1}, and at some $[\gamma\text{-CD}]_0$ and $[\text{Py}]_0$

Case	$[\text{Py}]_0$/M	$[\gamma\text{-CD}]_0$/M	Calculated [Py]/M	Calculated [γ-CD–pyrene]/M: Py·γ-CD	Py·Py·γ-CD	γ-CD·Py·γ-CD	γ-CD·Py·Py·γ-CD
1	5.0×10^{-7}	10^{-3}	3.21×10^{-7}	1.12×10^{-8}	6.84×10^{-8}	3.48×10^{-9}	1.39×10^{-8}
2		10^{-2}	8.52×10^{-8}	2.98×10^{-8}	4.83×10^{-8}	9.25×10^{-8}	9.79×10^{-8}
3	1.0×10^{-6}	10^{-3}	5.29×10^{-7}	1.85×10^{-8}	1.86×10^{-7}	5.74×10^{-9}	3.77×10^{-8}
4		10^{-2}	1.30×10^{-7}	4.56×10^{-8}	1.13×10^{-7}	1.41×10^{-7}	2.29×10^{-7}
5		5.0×10^{-2}	2.18×10^{-8}	3.81×10^{-8}	1.57×10^{-8}	5.90×10^{-7}	1.59×10^{-7}
6	1.0×10^{-5}	1.0×10^{-3}	2.20×10^{-6}	7.68×10^{-8}	3.20×10^{-6}	2.38×10^{-8}	6.49×10^{-7}
7		1.0×10^{-2}	4.69×10^{-7}	1.64×10^{-7}	1.46×10^{-6}	5.09×10^{-7}	2.97×10^{-6}
8		5.0×10^{-2}	9.78×10^{-8}	1.71×10^{-7}	3.18×10^{-7}	2.65×10^{-6}	3.22×10^{-6}
9	1.0×10^{-4}	1.0×10^{-2}	1.55×10^{-6}	5.41×10^{-7}	1.59×10^{-5}	1.68×10^{-6}	3.22×10^{-5}
10		5.0×10^{-2}	3.48×10^{-7}	6.09×10^{-7}	4.03×10^{-6}	9.44×10^{-6}	9.08×10^{-5}

or one of K_2 and K_4 were excluded, the steep decrease in intensity in excimer fluorescence at higher [γ-CD] could not be reproduced. The decrease in monomer fluorescence for 2mM $\leq$ [γ-CD] $\leq$ 10mM could not be recognized unless the contribution of an uncomplexed pyrene molecule was taken into account. Thus the summations of errors defined as the square of (observed fluorescence intensity minus calculated fluorescence intensity), $\Sigma(\delta_{\text{obs}} - \delta_{\text{calc}})^2$, were 0.049, 0.056, and 0.034 for cases I—III, respectively, except for points A, C, and D which deviate too much from the calculated intensities.

In the paper by Kano *et al.*,[14] the following set of *K* values was reported, K_1 20, K_2 5×10^6, and K_3 200 l mol^{-1}. We obtained the following sets of *K* values corresponding to the above cases I—III, respectively, by neglecting the intensity change on complexation (K_1 20, K_2 5.5×10^6, and K_3 200), (K_1 150, K_3 100, and K_4 2.0×10^6), and (K_1 20, K_2 4.5×10^6, K_3 300, and K_4 8.0×10^6 l mol^{-1}). Though $\Sigma(\delta_{\text{obs}} - \delta_{\text{calc}})^2$ values were larger than cases I—III above by an order of magnitude, still the error was the smallest when K_1—K_4 were all considered. In this way, the results of calculations suggest that complexation between pyrene and γ-CD takes place with stoicheiometries of 1 : 1, 1 : 2, 2 : 1, and 2 : 2. In particular, the anomalously large K_2 and K_4 values compared with those of K_1 and K_3 indicate that the second pyrene molecule can easily be trapped in the cavity of γ-CD. One of the most plausible reasons for this is ascribed to the space-regulating action[7] of the pyrene molecule first accommodated in γ-CD, since Corey–Pauling–Koltun (CPK) molecular models indicate that the inner cavity of γ-CD is large enough for two pyrene molecules. Of course, a concomitant increase in hydrophobicity cannot be ruled out.

The calculated concentrations of several γ-CD–pyrene complexes at the *K* values for curves c and c′ and for some $[\gamma\text{-CD}]_0$ and $[\text{Py}]_0$ are listed in the Table. As revealed, though 1 : 1, 1 : 2, 2 : 1, and 2 : 2 complexes are all formed in different ratios depending on both $[\gamma\text{-CD}]_0$ and $[\text{Py}]_0$, one of the most striking features is the finding that there exists a system which contains a 1 : 2 complex of γ-CD with pyrene alone.

Figure 3A shows the time variation of the fluorescence spectra of 1.0×10^{-5}M-pyrene in water in the presence of 1.0×10^{-2}M-γ-CD. When γ-CD was added to pyrene suspended in water (curve a), first the monomer fluorescence kept increasing for *ca.* 5 min and then changed as in the Figure, *i.e.*, the ratio of pyrene dimer increased with time. In contrast, if β-CD was used in place of γ-CD, mainly monomer fluorescence was observed.[13]

The time dependences of the excimer and monomer fluorescence intensities are shown in Figure 3B for solutions containing 1.0×10^{-5} or 10^{-4}M-pyrene after the addition of 1.0×10^{-2}M-γ-CD. Obviously, the decrease in monomer fluorescence was coupled with the increase in excimer fluorescence, suggesting that the 2 : 1 and 2 : 2 Py : γ-CD complexes can be formed from 1 : 1 Py : γ-CD complex. In contrast to 1 : 1 complexation between arenes and α- or β-CDs which occur almost instantly, it took several hours for γ-CD to solubilize two pyrene molecules in its cavity. The time required became shorter for higher [pyrene] solutions.

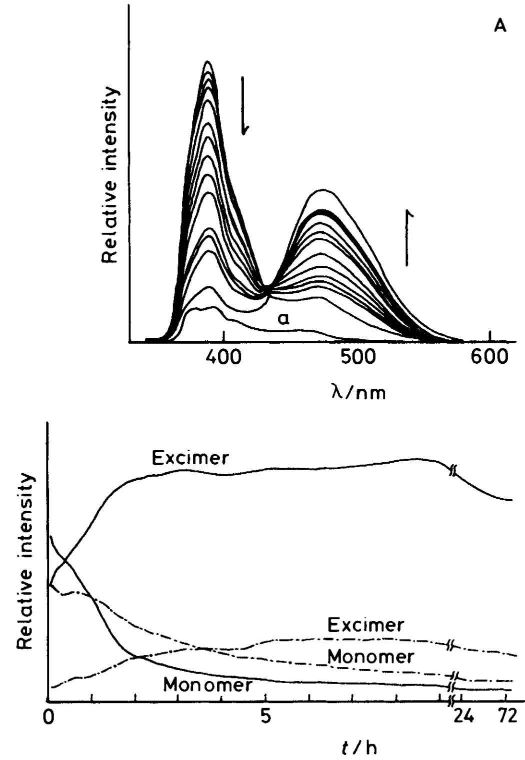

Figure 3. A, Time variation of the fluorescence spectra of 10^{-5}M-pyrene in water after the addition of 1.0×10^{-2}M-γ-CD. Changes in the initial 5 min have been eliminated. Curve a is an emission from 10^{-5}M-pyrene in the absence of γ-CD. B, Time dependencies of monomer and excimer fluorescence intensities of 10^{-4} (——) or 10^{-5}M-pyrene (— · — · —) after the addition of 1.0×10^{-2}M γ-CD

Figure 4 shows the absorption and c.d. spectra of 1×10^{-5}M-pyrene in water in the presence of 10^{-3}M-β- or γ-CD after ageing for >24 h. In the presence of β-CD, the signs of i.c.d. spectra were positive in the 245—270 and 290—360 nm regions and negative in the 275—290 nm region. In addition, the positions of the peaks and the trough were in complete accord with those of the absorption peaks. Concerning the

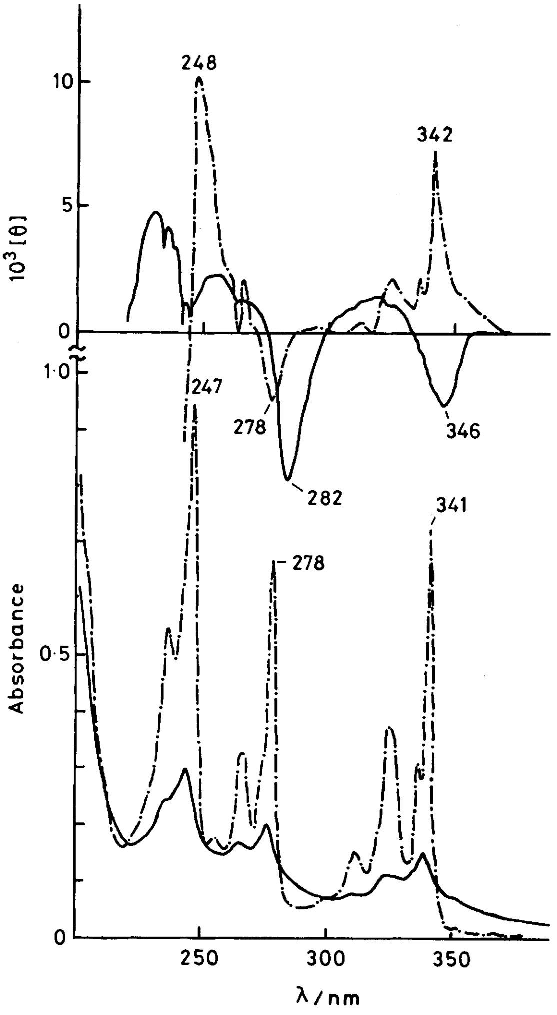

Figure 4. Absorption (bottom) and i.c.d. spectra (top) of 10^{-5}M-pyrene in water in the presence of 5.0 × 10^{-3}M-β- (— • — • —) or γ-CD (——). The temperature was *ca.* 20 °C. Path lengths were 100 mm and 10 mm for i.c.d. and absorption spectra, respectively

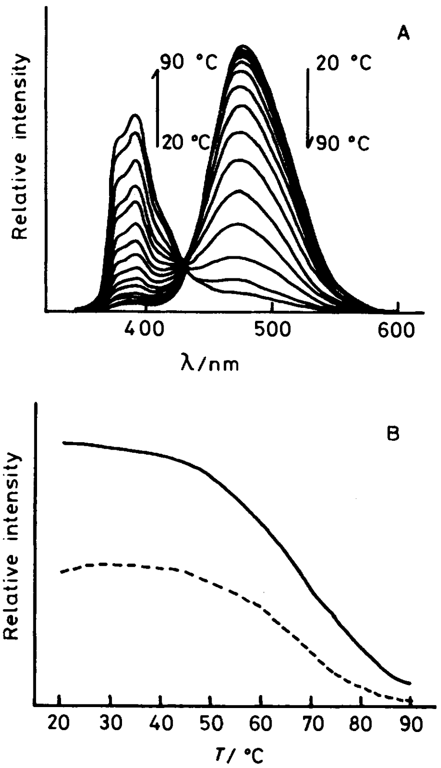

Figure 5. A, The temperature dependence of the fluorescence spectrum of the matured solution containing 10^{-4}M-pyrene and 1.0 × 10^{-2}M-γ-CD. The temperature was regulated by a Haake F2 thermostat, and kept constant for at least for 1 h at each temperature prior to measurements (separate experiments indicated that 30 min was enough to obtain a stable spectrum). **B**, The intensity change of excimer fluorescence with temperature for solutions containing 10^{-4}- (——) or 10^{-5}M-pyrene (- - - -) and 1.0 × 10^{-2}M-γ-CD

sign of the i.c.d. spectra of arenes included in β-CD, it has already been substantiated, on the basis of the Kirkwood–Tinoco coupled oscillator expression, that a positive sign is produced by the electronic transitions parallel to the molecular axis of β-CD.[17,18] Since the spectrum of pyrene is polarized along the long axis in the 290—360 nm region,[19] the above i.c.d. result suggests an axial inclusion in which the long axis of pyrene is parallel to the axis of γ-CD. In contrast, in the presence of γ-CD, two sinusoidal i.c.d. curves with a change in sign from minus to plus from the longer wavelength side were observed, associated with the ${}^1L_a$ and ${}^1B_b$ transitions, suggesting inclusion of more than one pyrene molecule. Since the CPK molecular model effectively eliminates the possibility of inclusion of more than two pyrene molecules, and the results in Table 1 reveal that the major species is a 1 : 2 γ-CD–pyrene complex under present conditions, the i.c.d. spectrum in Figure 4 seems most conclusively to be that of γ-CD containing two pyrene molecules. Thus, the configuration of two pyrene molecules included is an *S*-helix due to the change of sign in the i.c.d. spectrum at the ${}^1L_a$ band.

The temperature dependence of the fluorescence spectrum of an aged solution containing 1.0 × 10^{-4}M-pyrene and 1.0 × 10^{-2}M-γ-CD is illustrated in Figure 5A. At ambient temperature, the spectrum consists mainly of excimer fluorescence. With a rise in temperature, the intensity of excimer fluorescence decreased, while that of monomer fluorescence increased concomitantly with an isoemissive point at 432 nm, and finally at 90 °C the spectrum became practically that of monomer fluorescence. I.c.d. spectra were also measured at higher temperatures. With a rise in temperature, the intensity of the trough at 346 nm observed at room temperature (Figure 4) diminished and above 80 °C the spectral pattern accorded approximately with that of the β-CD–pyrene system (Figure 4) except for a 6 nm blue-shift (Figure 6), indicating a formation of a 1 : 1 axial inclusion complex.* Thus, this spec-

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* The estimate in the computer simulation of [γ-CD] *versus* the fluorescence intensity plots that the quantum yields of excimer fluorescence of Py·Py·γ-CD and/or γ-CD·Py·Py·γ-CD complexes are *ca.* 1.5 times larger than those of the monomer fluoresence of Py·γ-CD and/or γ-CD·Py·γ-CD complexes originates for observations of Figures 5A and 6 as follows. As revealed in Figure 5A, the solution which shows virtually only excimer fluorescence alone at room temperature changes to give only monomer fluorescence at 90 °C. In addition, an isoemissive point is observed during this change. If species such as Py, Py·γ-CD, and γ-CD·Py·γ-CD are all formed at higher temperatures, an isoemissive point cannot be detected except when their quantum yields of monomer fluorescence are the same. Since the quantum yield of monomer fluoresence of Py differs from that of Py·γ-CD and/or γ-CD·Py·γ-CD, however, not all these species can be present at higher temperatures. That is, there seems to be a predominant species at higher temperatures. This species seems to be a 1 : 1 Py : γ-CD axial inclusion complex

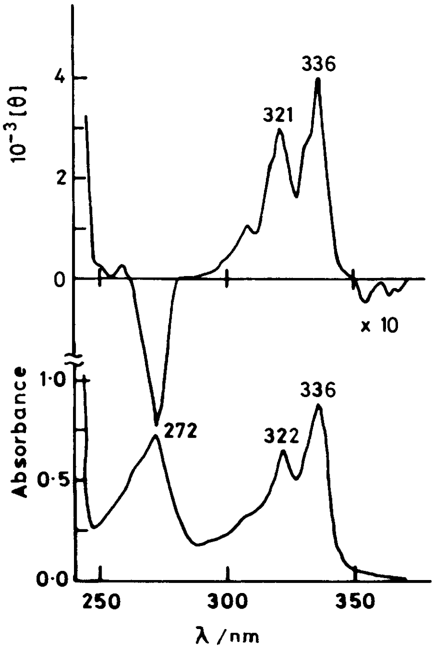

Figure 6. Absorption (bottom) and i.c.d. spectra (top) of 10^{-4}M-pyrene in pH 7.02 phosphate buffer in the presence of 1.0×10^{-2}M-γ-CD. The temperature was 85 °C and the path length 10 mm

tral behaviour reveals that one of the two pyrene molecules trapped in γ-CD at ambient temperature can be transferred to the solution at higher temperatures.

Figure 5B displays the temperature dependence of the excimer fluorescence intensity for solutions containing 1.0×10^{-4} or 10^{-5}M-pyrene and 1.0×10^{-2}M-γ-CD. The transformation of a pyrene dimer into a monomer occurred at *ca.* 66 °C, though the values of K_2/K_1, K_4/K_1, K_2/K_3, and K_4/K_3 which can be obtained for the calculated K values seem a little too large for such a phenomenon to take place at this temperature.

In conclusion, we have shown that γ-CD can form inclusion complexes with pyrene molecules in ratios of 1 : 1, 1 : 2, 2 : 1, and 2 : 2. The inclusion of two pyrene molecules by γ-CD proceeds stepwise, and their configuration in the cavity is that of an *S*-helix. One of the two pyrene molecules is removed from the γ-CD cavity at higher temperature to yield a 1 : 1 γ-CD–pyrene axial inclusion complex.

Acknowledgements

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from the i.c.d. spectra in Figure 6. Then, assuming roughly that all Py·Py·γ-CD and γ-CD·Py·Py·γ-CD at room temperature change into Py·γ-CD (plus a little γ-CD·Py·γ-CD) at 90 °C, the quantum yields of excimer fluorescence of Py·Py·γ-CD and/or γ-CD·Py·Py·γ-CD are *ca.* 1.5 times larger than those of monomer fluorescence of Py·γ-CD and/or γ-CD·Py·γ-CD by comparing the excimer fluorescence intensity at 20 °C with the monomer fluorescence intensity at 90 °C in Figure 5A.