Evidence for Highly Selective Supramolecular Formation between Perylene/γ-CD and Pyrene/γ-CD Complexes in Water

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We have studied the simultaneous complexation of pyrene and perylene with γ-cyclodextrin in water by means of electronic excitation energy transfer. We found evidence that although in the solution there are present three different pyrene/γ-cyclodextrin complexes with stoichiometries of 1:1, 1:2, and 2:2 the (perylene)$_1$/(γ-cyclodextrin)$_2$ complex that is formed associates exclusively with the excimer-emitting (pyrene)$_2$/(γ-cyclodextrin)$_2$ adduct and not at all with the others. Moreover, this effect is not observed when pyrene is replaced by some of its homologues, which also form 2:2 excimer-emitting complexes with γ-cyclodextrin.

Introduction

Complexation with organic molecules in aqueous media is one of the most remarkable properties of cyclodextrins (CDs). Provided guest molecules have dimensions compatible with the cyclodextrin cavity, weak adducts can be formed, which usually demonstrate simple guest/CD stoichiometries, primarily 1:1, 1:2, and 2:2.[1] In addition, supramolecular assemblies, which involve several cyclodextrin units and guest molecules such as catenates, rotaxanes, polyrotaxanes, threaded cyclodextrins,[2] nanotubes,[3,4] and so forth, can also result. For all of these complexes, simple and supramolecular, that are held together by noncovalent chemical bonds, fluorescence spectroscopy, both steady state and time-resolved, is among the most appropriate methods of investigation.[5]

In the present study, our aim was to examine the simultaneous complexation of pyrene (py) and perylene (pe) with γ-cyclodextrin (γ-CD) in water. The adduct formation between pyrene and γ-CD in pure water has been studied at length, and the stoichiometries and equilibrium constants of the formed complexes are well known.[6−8] Therefore, we have adopted published data,[6] which we have also confirmed by our own measurements. Contrarily, the adduct formation between perylene and γ-CD in water has attracted very little attention,[9] evidently because of the very low solubility of this fluorophore in water (ca. 1.6 × 10^{-9} M);[10] therefore, we had to carry out our own preliminary study.
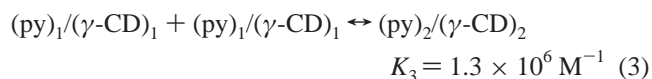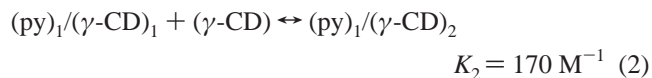
Experimental Part

Pyrene (Aldrich 99%) was purified by zone refining, perylene (Aldrich 99%+ Gold label) was used without any treatment, and γ-CD was purchased from Cyclolab and was used as received. The instruments and methods employed in this study have been previously described.[3]

Two totally different methods were employed for the preparation of the solutions. In the first, pyrene was deposited by evaporation from an ethanolic solution on the wall of a beaker, and then a 10^{-2} M aqueous solution of γ-CD was added and left overnight to solubilize the fluorophore. In another beaker,

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perylene was likewise deposited on the wall by evaporation from a solution in ethanol. To this second beaker the previously prepared solution of pyrene in γ-CD was added and left overnight to solubilize perylene. According to the second method, a few microliters of an appropriate solution of pyrene in ethanol was injected into an aqueous solution of 10^{-2} M γ-CD; to the resulting solution, a few microliters of perylene in ethanol or dioxane was injected, thus forming the final aqueous solution of pyrene + perylene in γ-CD. In both cases, the concentrations of pyrene and perylene solubilized in 10^{-2} M aqueous γ-CD were determined from their absorption spectra using $\epsilon_{335} = 54\,000$ for the former and $\epsilon_{438} = 42\,100$ for the latter. Note that in the case of pyrene, because of the dimerization in (py$_2$)/(γ-CD) and the change in the absorption spectra, the absorption was measured after NaOH was added to break the dimers.[11,12] Solutions prepared by either method gave, within experimental error, identical steady-state and time-resolved fluorescence results.

Results and Discussion

Pyrene/γ-CD in Water. Pyrene/γ-CD complexation in water has been studied extensively by several authors.[6−8] It is well established[6] that three different complexes are formed, viz., (py)$_1$/(γ-CD)$_1$, (py)$_1$/(γ-CD)$_2$, and (py)$_2$/(γ-CD)$_2$, according to eqs 1−3.

$$\text{py} + \gamma\text{-CD} \leftrightarrow (\text{py})_1/(\gamma\text{-CD})_1 \qquad K_1 = 300\ \text{M}^{-1} \quad (1)$$

$$(\text{py})_1/(\gamma\text{-CD})_1 + (\gamma\text{-CD}) \leftrightarrow (\text{py})_1/(\gamma\text{-CD})_2$$
$$K_2 = 170\ \text{M}^{-1} \quad (2)$$

$$(\text{py})_1/(\gamma\text{-CD})_1 + (\text{py})_1/(\gamma\text{-CD})_1 \leftrightarrow (\text{py})_2/(\gamma\text{-CD})_2$$
$$K_3 = 1.3 \times 10^6\ \text{M}^{-1} \quad (3)$$

The first two complexes emit monomer pyrene fluorescence, and the third emits typical excimer fluorescence. Figure 1 shows the concentrations of the complexes formed in aqueous solutions containing [py] = 2.6 × 10^{-6} M and [γ-CD] = 0−0.01 M. Note that the concentrations of the complexes do not add up to the analytical concentration of pyrene because (py)$_2$/(γ-CD)$_2$ contains two pyrene molecules.

Perylene/γ-CD and Pyrene/γ-CD Complexes in Water

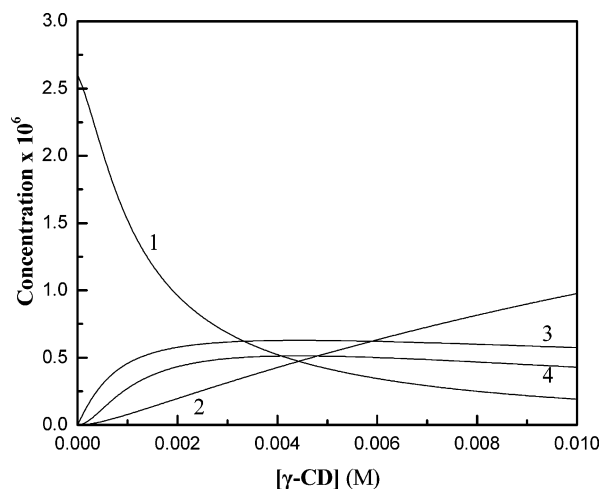*J. Phys. Chem. B, Vol. 108, No. 9, 2004* **2847**

Figure 1. Concentrations of the various species present in an aqueous solution containing 2.6×10^{-6} M pyrene and various amounts of γ-CD. (1) Free pyrene. (2) (py)$_1$/(γ-CD)$_2$. (3) (py)$_2$/(γ-CD)$_2$. (4) (py)$_1$/(γ-CD)$_1$.

Perylene/γ-CD in Water. As we have already mentioned, perylene has extremely low solubility in water. For this reason, there are not any systematic studies of its complexation with γ-CD in water. Nevertheless, evidence based on fluorescence data has been published that suggests that the 1:2 complex of (pe)$_1$/(γ-CD)$_2$ is formed in pure water.[9] We have confirmed this finding by means of steady-state fluorescence anisotropy and the Perrin−Einstein equation (eq 4).

$$\frac{1}{r_{ss}} = \frac{1}{r_0} + \frac{\tau R T}{r_0 \eta V} \tag{4}$$

In this equation, r_{ss} is the steady-state fluorescence anisotropy of perylene in the adduct (we measured $r_{ss} = 0.054$), r_0 is the value of the fluorescence anisotropy of perylene in a frozen solution (we measured $r_0 = 0.38$), τ is the fluorescence lifetime of perylene in the adduct (we measured $\tau = 5.6$ ns), R is the gas constant, the temperature is $T = 298$ K, and $\eta = 0.01$P is the viscosity of water. From the above, we have calculated the hydrodynamic volume V of the rotating adduct equal to ca. 2300 cm^3/mol, which agrees quite well with the volume of two γ-CD units, viz., $2 \times 1095 = 2190$ cm^3/mol.[13] We have concluded therefore that the complexation of perylene with γ-CD in water indeed involves the formation of the barrel-type species (pe)$_1$/(γ-CD)$_2$, in which perylene is enclosed inside the cavity formed by the two γ-CD units.

Pyrene/Perylene/γ-DC in Water. When two pyrene molecules are entrapped in a restricted space, such as the cavity formed by two cyclodextrin units, they form loosely bound ground-state dimers, which emit excimer fluorescence.[6] The excitation spectra of such dimers can be easily obtained if the fluorescence is monitored at some wavelength where only the excimer emits. When the fluorescence is monitored at a wavelength where only the pyrene monomer emits, one obtains the excitation spectrum of monomeric pyrene. Such excitation spectra of an aqueous solution containing 2.6×10^{-6} M pyrene and 10^{-2} M γ-CD are shown in Figure 2. In one spectrum (solid line), the excitation was monitored at 480 nm where only pyrene excimer emits; therefore, this spectrum corresponds to the dimerized pyrene, viz., to the (py)$_2$/(γ-CD)$_2$ complex. The other spectrum of Figure 2 (dashed line) was obtained with the excitation monitored at 373 nm where only monomeric pyrene emits; therefore, this spectrum corresponds to the pyrene monomers present in the solution, viz., (py)$_1$/(γ-CD)$_1$, (py)$_1$/(γ-CD)$_2$, and free pyrene molecules.
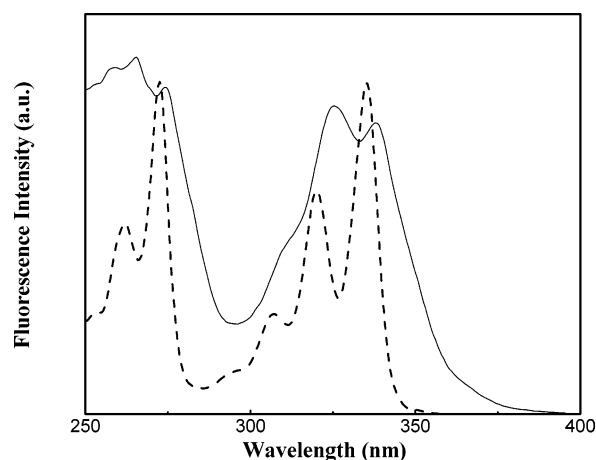

Figure 2. Excitation spectra of 2.6×10^{-6} M pyrene/10^{-2}M γ-CD in water. (− − −) emission monitored at 373 nm; the spectrum corresponds to monomeric pyrene, viz., (py)$_1$/(γ-CD)$_1$, (py)$_1$/(γ-CD)$_2$ and free py. (—) Emission monitored at 480 nm; the spectrum corresponds only to (py)$_2$/(γ-CD)$_2$.
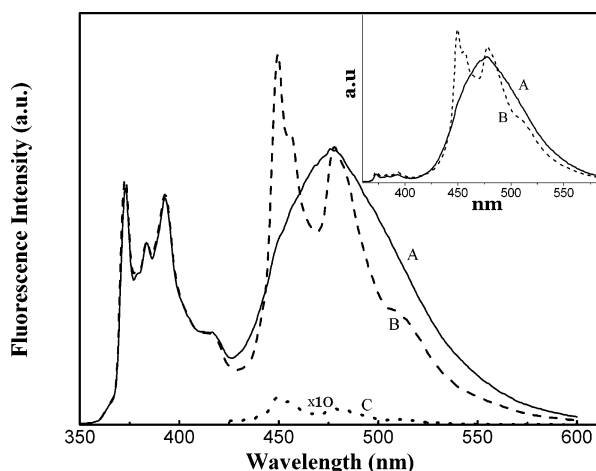

Figure 3. Fluorescence spectra excited at 338 nm. (A) Emission of 2.6×10^{-6} M pyrene/10^{-2} M γ-CD in water. (B) Same as in part A but with 1×10^{-7} M perylene added. (C) Emission of 1×10^{-7} M perylene/10^{-2} M γ-CD in water. (Inset) Emission spectra that are the same as in A and B but obtained by direct excitation of the dimer at 350 nm.

In Figure 3, spectrum A (solid line) depicts the total fluorescence spectrum of the above solution (2.6×10^{-6} M pyrene, 10^{-2} M γ-CD in water) with excitation at 338 nm, where both the monomeric and the dimerized pyrene molecules absorb to approximately the same extent (Figure 2). The two fluorescence spectra are clearly distinguished, with the excimer centered around 480 nm and the monomer centered around 380 nm. Note that when an aqueous solution of 1×10^{-7} M perylene and 10^{-2} M γ-CD was excited at the same wavelength—338 nm—a hardly perceptible perylene emission spectrum was detected, viz., spectrum C (dotted line) of Figure 3. (Note the intensity multiplication factor of 10.) This was expected in view of the extremely weak absorption of perylene at 338 nm.[14]

However, when the above two solutions were appropriately mixed in such a way as to keep all concentrations the same, viz., [py] = 2.6×10^{-6} M, [pe] = 1×10^{-7}M, and [γ-CD] = 10^{-2} M, excitation at 338 nm produced very strong perylene fluorescence (dashed line in Figure 3).

When comparing the spectra of Figure 3, it becomes evident that in the presence of pyrene the fluorescence of perylene is strongly sensitized. The only explanation for this sensitization is that in the mixed solution electronic excitation energy is

SCHEME 1
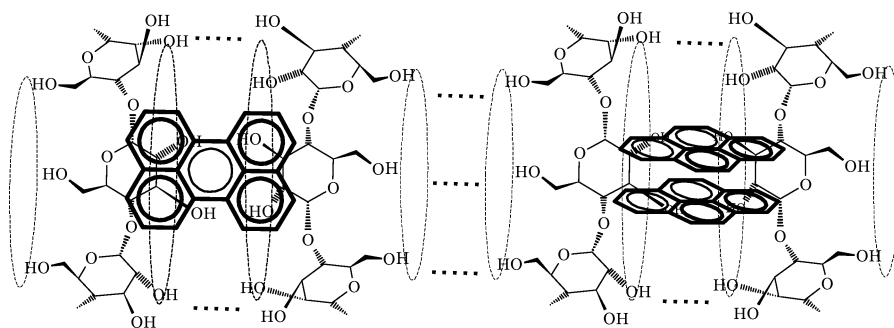
transferred from pyrene to perylene. In view of the very low concentrations of the two fluorophores (10^{-6}–10^{-7}M), it is surprising to observe energy transfer unless some sort of association takes place between the donor and acceptor molecules, bringing them close to each other (up to ca. 100 Å for resonance-energy transfer to occur).

The very interesting aspect of this sensitization is that only energy from the excimer-emitting pyrene dimer, viz., $(py)_2/(\gamma\text{-}CD)_2$, is transferred to perylene. Indeed, the spectra of Figure 3 (dashed and solid lines) clearly show that although part of the intensity of the excimer pyrene fluorescence has been replaced by the emission of perylene the monomer fluorescence of pyrene was not affected in the least. Similar results were obtained when the above solution was excited by 350-nm light, which is absorbed only by the pyrene dimer in the complex $(py)_2/(\gamma\text{-}CD)_2$ (inset of Figure 3). Moreover, excitation spectra of the same solution, obtained by monitoring the emission at one of the peaks of the fluorescence of perylene (e.g., 480 nm) produced the excitation spectrum of the dimerized pyrene in $(py)_2/(\gamma\text{-}CD)_2$ (solid line of Figure 2), confirming that all of the sensitization of perylene occurs via energy transfer from this particular species. It is therefore certain that energy transfer takes place only from the excimer-emitting $(py)_2/(\gamma\text{-}CD)_2$ complex and not at all from the pyrene-monomer-emitting species present in the solution. Note that according to Figure 1, at the concentrations of our experiments, viz., [py] = 2.6×10^{-6} M and [γ-CD] = 10^{-2} M, there are more than twice as many species involving monomeric rather than dimerized pyrene. Moreover, at the concentrations used in our experiments (1×10^{-7} M perylene and 2.6×10^{-6} M pyrene), the concentration of perylene is high enough for it to associate only with ca. 10% of $(py)_2/(\gamma\text{-}CD)_2$, whose concentration (from Figure 1) is 1×10^{-6} M. Therefore, only about 10% of the excimer pyrene fluorescence will have a probability of being transferred to perylene; the remaining 90% of $(py)_2/(\gamma\text{-}CD)_2$ will emit its ordinary excimer fluorescence. Consequently, the sensitized spectrum of perylene (spectrum B in Figure 3 and the inset) will be superimposed on the excimer spectrum originating from the $(py)_2/(\gamma\text{-}CD)_2$ complexes that are not associated with perylene. This is exactly what is observed as shown in Figure 3 and the inset, where the sensitized fluorescence of perylene lies on top of a broad background, which corresponds to the excimer emission of pyrene. When, however, pyrene was diluted in the solution, keeping both [γ-CD] and [pe] constant at 10^{-2} and 1×10^{-7}M, respectively, the shape of the fluorescence spectrum of perylene, obtained by energy transfer (excitation at 338 nm), was identical to the spectrum obtained by direct excitation at 430 nm without the broad excimer emission underneath. It should be emphasized here that although in the above solution, viz., py/pe/γ-CD/water, we did not observe any energy transfer from the monomeric pyrene to perylene, under isotropic conditions transfer from monomeric

pyrene to perylene is known to occur quite efficiently with a corresponding characteristic Förster distance, R_0 = 33.3 Å.[15] Recall that R_0 is the donor–acceptor distance at which 50% of the donor molecules decay by energy transfer and 50% decay by the usual radiative or nonradiative processes.

The above results clearly lead to the conclusion that in aqueous solutions the excimer-emitting $(py)_2/(\gamma\text{-}CD)_2$ complex and perylene are brought together, with the consequence that excitation energy is transferred from the former to the latter. There are two conceivable ways in which perylene can attach itself to the barrel-type $(py)_2/(\gamma\text{-}CD)_2$ complex: either as a free perylene molecule or as $(pe)_1/(\gamma\text{-}CD)_2$, which forms when perylene interacts with γ-CD in water. In the first case, a perylene molecule enters the cavity formed by the two γ-CD units, where it resides in close proximity to the pyrene dimer. Should that be the case, one would have expected to see some changes in the excitation spectrum of the pyrene dimer because of the proximity of the perylene molecule; however, such changes were not observed. Moreover, the energy transfer from the pyrene excimer to perylene should be very fast; therefore, the decay of the fluorescence of perylene excited via energy transfer should not exhibit any rise time. Contrary to this, as we discuss in the following paragraph, a rise time of 2 ns is observed when perylene is excited by energy transfer. The experimental evidence therefore does not agree with the association of free perylene molecules with the excimer-emitting $(py)_2/(\gamma\text{-}CD)_2$ complex. In the second alternative, viz., when a superstructure such as $(\gamma\text{-}CD)/(py_2)/(\gamma\text{-}CD)/(\gamma\text{-}CD)/(pe)/(\gamma\text{-}CD)$ is formed, the distance between perylene and the pyrene dimer should be equal to approximately twice the height of one γ-CD unit (Scheme 1). It is crucial, therefore, to determine the separation between perylene and the pyrene dimer at which the observed energy transfer occurs. For this, we have used data obtained from the time-resolved study of the energy transfer.

Thus, we have observed a rise time of $\tau_r = 2 \pm 0.5$ ns for the decay of perylene when it was excited through energy transfer from $(py)_2/(\gamma\text{-}CD)_2$ (i.e., excitation at 350 nm, decay monitored at 480 nm; Figure 4). Contrarily, in the absence of perylene we did not observe any such rise in the decay profile of the pyrene dimer (Figure 4), in agreement with published results.[16] The measured rise time allows the determination of the energy-transfer efficiency E_{tr}, through the relation

$$E_{tr} = \frac{1}{\tau_r}\left(\frac{1}{\tau_d} + \frac{1}{\tau_r}\right) \tag{5}$$

where τ_d is the lifetime of the donor in the absence of an acceptor and is equal to 70 ns in the present case. The energy-transfer efficiency thus estimated was found to be equal to 97%. Furthermore, from the fluorescence quantum yield Q_d of the donor (pyrene dimer) in the absence of the acceptor (perylene), which we found to be equal to 0.67, and the other parameters
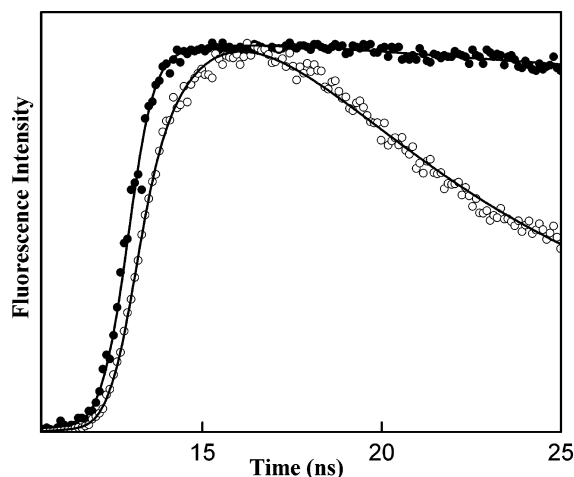
Figure 4. Fluorescence decay profiles (linear scale) of perylene excited via energy transfer from dimeric pyrene (○) and decay of the pyrene excimer alone (●). In both cases, excitation was at 350 nm, and emission was monitored at 480 nm.

involved in the resonance-energy transfer (eq 6[17])

$$R_0 = 0.211\{\kappa^2 \eta^{-4} Q_d J(\lambda)\}^{1/6} \qquad (6)$$

we have calculated $R_0 = 29$ Å. Note that in this equation κ is an orientational parameter for which the value of $\kappa^2 = {}^2/_3$ is used, η is the refractive index of the solvent, and $J(\lambda)$ is the overlap integral that expresses the degree of spectral overlap between the donor emission and acceptor absorption.[17] Next, using the above τ_r and R_0 values and the relationship

$$\frac{\tau_d}{\tau_r} = \left(\frac{R_0}{R}\right)^6 \qquad (7)$$

of the Förster theory, we have estimated the donor−acceptor mean distance to be equal to $R = 16$ Å. This length is very close to the length of two γ-CD units placed next to each other, rim to rim (7.9 Å per CD[13]), and therefore supports quite accurately the formation of the supramolecular assembly (γ-CD)/(py$_2$)/(γ-CD)/(γ-CD)/(pe)/(γ-CD) mentioned earlier. Note that this donor−acceptor separation, $R = 16$ Å, was not affected when the concentrations of perylene and pyrene were reduced in three steps, from [py] $= 2.6 \times 10^{-6}$ M, [pe] $= 1 \times 10^{-7}$ M down to [py] $= 3.2 \times 10^{-7}$ M, [pe] $= 2.5 \times 10^{-8}$M, while keeping the concentration of γ-CD constant at 10^{-2} M. Indeed, the rise time of the energy-transfer-induced fluorescence of perylene was kept the same, viz., 2 ± 0.5 ns, over these entire pyrene and perylene concentration ranges. This shows that adduct formation is not affected by the comparatively large concentration of γ-CD.

Because of the very low concentration of the formed supramolecular structure, $<10^{-7}$M, we were unable to determine with certainty the fluorescence anisotropy of perylene in the large assembly; therefore, we do not have reliable estimates of its size. However, a value in the range of $r_{ss} = 0.1−0.12$, which we found for the fluorescence anisotropy when perylene was directly excited, indicates that, according to the Perrin−Einstein equation (eq 4), the formed supramolecular structure is made of approximately four to five γ-CD units. A reasonable visualization of the formed superstructure, which is not in disaccord with our experimental findings, is depicted in Scheme 1. The assembly is shown as occurring by association, probably through hydrogen bonding, of the two complexes across the narrower rims of two γ-CD units, one from each complex.

Finally, we have examined some pyrene derivatives which that are also known to form excimer-emitting barrel-type 2:2 complexes with γ-CD in water, viz., 1-methylpyrene,[18] sodium 1-pyrenesulfonate,[19] and sodium 1-pyrenebutyrate.[20] In all of these cases, we found that when the (pe)/(γ-CD)$_2$ complex was added to the aqueous solution of the 2.6×10^{-6} M pyrene derivative and 10^{-2} M γ-CD energy transfer from pyrene to perylene did not occur either in the form of monomeric or excimeric excitation. On the contrary, under isotropic conditions we found that these pyrene derivatives transfer energy to perylene at rates comparable to those of pure pyrene, $R_0 = 30−35$ Å. These findings show that the large supramolecular assembly formed in the system (py)$_2$/(γ-CD)$_2$/(pe)$_1$/(γ-CD)$_2$ is unique. Admittedly, this selective association of the particular adducts is a very strange effect; nevertheless, it has been well documented by the experimental evidence presented here. Regarding its origin, one would be inclined to suggest that differences in the hydrogen bonding, which is mainly responsible for rim-to-rim cyclodextrin interactions, are the cause of the differences observed here. However, it is not at all clear why the intercyclodextrin hydrogen bonds should be affected to such a great extent by the nature of the guest molecule.

Furthermore, the distance between the pyrene excimer and perylene in the (py)$_2$/(γ-CD)$_2$/(pe)$_1$/(γ-CD)$_2$ supramolecule is very large, which allows some contribution from the specific interactions that have been reported to occur between perylene and pyrene's excimer in different media.[21] Therefore, presently we cannot offer a satisfactory explanation for this selective supramolecular association, and it is best to avoid speculation. One last comment is in order here: it is very likely that similar highly selective interactions may occur between other cyclodextrin adducts, but they have not been detected because of the fact that appropriate sensitive techniques such as energy transfer are not applicable.

Conclusions

The main conclusion of the present study is that in aqueous solutions of pyrene, perylene, and γ-CD the excimer-emitting complex (py)$_2$/(γ-CD)$_2$ associates with (pe)$_1$/(γ-CD)$_2$, as inferred from the study of electronic excitation energy transfer. Although two more complexes of pyrene are present in the solution, viz., (py)$_1$/(γ-CD)$_1$ and (py)$_1$/(γ-CD)$_2$, none of them associates with (pe)$_1$/(γ-CD)$_2$. Moreover, this association is characteristic of the particular system pyrene/perylene/γ-CD/water because it ceases to occur when pyrene is replaced by some of its derivatives, which have similar complexation properties to those of γ-CD, suggesting that the supramolecular structure discussed here is of a unique nature indeed. Presently, we do not have a complete and satisfactory explanation for this effect.

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