Study of Poly(propylene imine) Dendrimers in Water, by Exciplex Formation

G. Pistolis and Angelos Malliaris*

NCPS "Demokritos", Athens 15310, Greece

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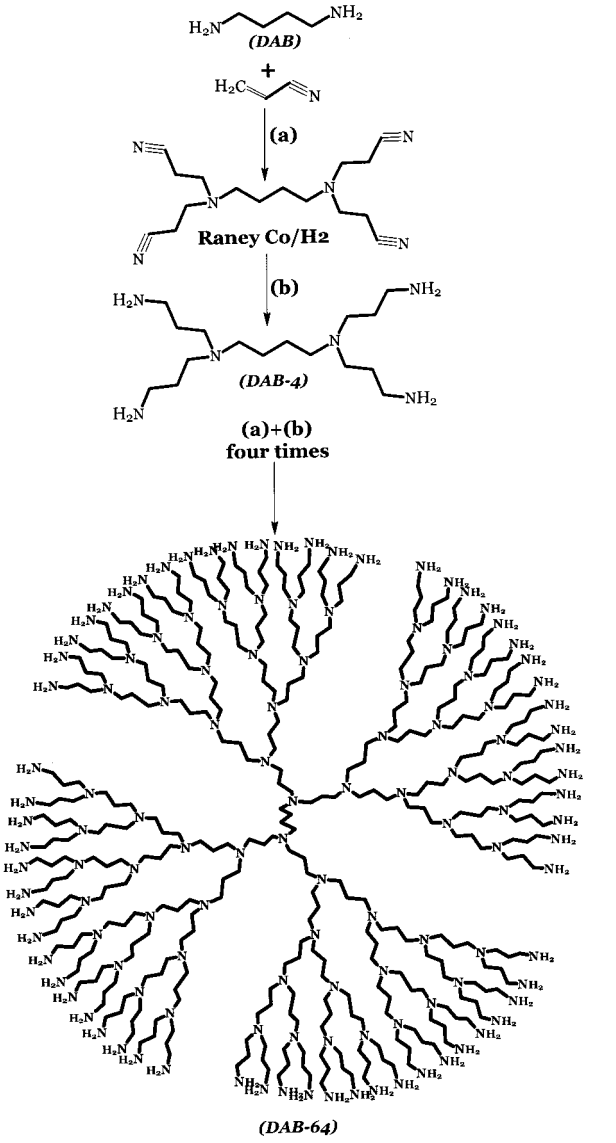
Static and time-resolved fluorescence methods were employed to evaluate the various generations of poly(propyleneimine) dendrimers as solubilizing agents for hydrophobic molecules in aqueous media. In addition, the penetration of water inside the dendritic core was studied by means of the formation of emissive exciplexes between pyrene and the tertiary amine groups at the branching points of these dendrimers.

I. Introduction

Dendrimers are hyperbranched polymers consisting of a central core and a number of monomeric repeat units which bear appropriate functional groups, e.g., CN, NH_2, etc.[1−5] From the synthetic point of view, the primary function of these groups is to act as branching points for the expansion of the dendritic molecular unit. The basic outline of the divergent stepwise synthesis of the particular dendrimer studied here, namely, poly(propylene imine), is depicted in Scheme 1, where the 1,4-diaminobutane (DAB) is used as the core, while the repeat unit is the propylamine group $-CH_2-CH_2-CH_2-NH_2$. Therefore the various generations of this dendrimer consist of the DAB core to which four branched poly(propylene amine) structures are attached. We have labeled these dendrimers with the name of the core (DAB) followed by a numeral indicating the number of NH_2 end groups, e.g., DAB-4, DAB-32, etc. Therefore, the periphery of the dendritic unit is filled with primary amine groups, available for further growth of the polymer, while all the branching points, in the interior of the dendrimer, are occupied by tertiary nitrogens, which are well-known effective quenchers of the fluorescence of pyrene, via exciplex formation.[6−8]

Following up on our previous work concerning the pH-sensitive controlled release of pyrene from DAB-32 and DAB-64 dendrimers in water,[9] we have examined the static and time-resolved spectroscopic behavior of pyrene in aqueous solutions of DAB dendrimers. Our aim was to obtain information about the interior of these supramolecules, particularly with respect to the water penetration and the ensuing polarity. To this end we employed the fluorescent probing technique with pyrene as fluorophore. Previous studies on DAB dendrimers with various end groups have been reported involving small angle neutron scattering (SANS),[10,11,12,13] low angle laser light scattering

Scheme 1



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(LALLS),[14] vapor pressure osmometry,[14] viscosimetry,[13,14] molecular dynamic simulations,[13] etc.

II. Experimental Section

The dendrimers DAB-4, DAB-16, DAB-32, and DAB-64 were purchased from DSM Fine Chemicals Company and were used without further purification. The DAB-8 homologue was found impure and therefore it was not used in this study. (Note that the lack of data for DAB-8 is not very crucial for the present study since true dendrimer behavior is expected only from the highest homologues, DAB-32 and DAB-64.) Pyrene (Aldrich, >99%) was purified by zone refining, whereas triethanolamine (Carlo Erba, >99.5%) was used as received. Absorption spectra were obtained with a Perkin-Elmer Lamba-16 spectrophotometer, while for recording steady-state fluorescence spectra we used the Perkin-Elmer Model LS-50B and the Edinburgh Instruments Model FS-900 spectrofluorometer. Time-resolved fluorescence measurements were carried out with the single-photon counter, FL900, of the above Edinburgh Instruments setup. Some background emission from the aqueous solutions of the dendrimers was always subtracted from the fluorescence of the samples. More details about the experimental methods used in the present study are described in previous reports.[15]

III. Results and Discussion

The tertiary amine group, which marks each branching point of a DAB dendrimer, is a well-known quencher of the fluorescence of pyrene.[6,8] In view of this and also of pyrene's hydrophobicity, which drives Py molecules inside the dendrimers where the effective concentration of tertiary nitrogens is very high (30−50 M/L), we expect the binding between pyrene and DAB to be readily determined through the decrease of the fluorescence intensity when an aqueous solution of pyrene is titrated with aqueous solutions of various generations of DAB dendrimers.

Probe Solubilization. In Figure 1a, the ratio F/F_o is plotted vs the concentration of added dendrimer, where F_o stands for the fluorescence intensity of pyrene dissolved in water and F for the intensity after each successive addition of dendrimer. If we assume that the pyrene/dendrimer complexation follows a 1:1 stoichiometry, i.e.,

$$\text{Py} + \text{DAB} \overset{K_b}{\leftrightarrow} \text{Py-DAB}$$

then the binding constant K_b is given by eq 1,

$$K_b = [\text{Py-DAB}]/[\text{Py}][\text{DAB}] \quad (1)$$

where [Py-DAB], [Py], and [DAB] are the concentrations of the complex formed and of the unbound fluorophore and dendrimer, respectively. On the other hand, to maintain the mass balance between reactants and reaction products, eq 2 must also be satisfied, where $[\text{Py}_o]$ is the

$$[\text{Py}_o] = [\text{Py}] + [\text{Py-DAB}] \quad (2)$$

analytical concentration of pyrene before any dendrimer was added. Combining eqs 1 and 2 we obtained eq 3, while by introducing into it the molar fractions of the bound,

$$[\text{Py-DAB}]/[\text{Py}_o] = K_b[\text{DAB}]/(1 + K_b[\text{DAB}]) \quad (3)$$

$f_b = [\text{Py-DAB}]/[\text{Py}_o]$, and the free, $f_f = [\text{Py}]/[\text{Py}_o]$, pyrene and taking into account that $f_b + f_f = 1$, we ended up with eq 4. This equation gives F in terms of the molar fractions

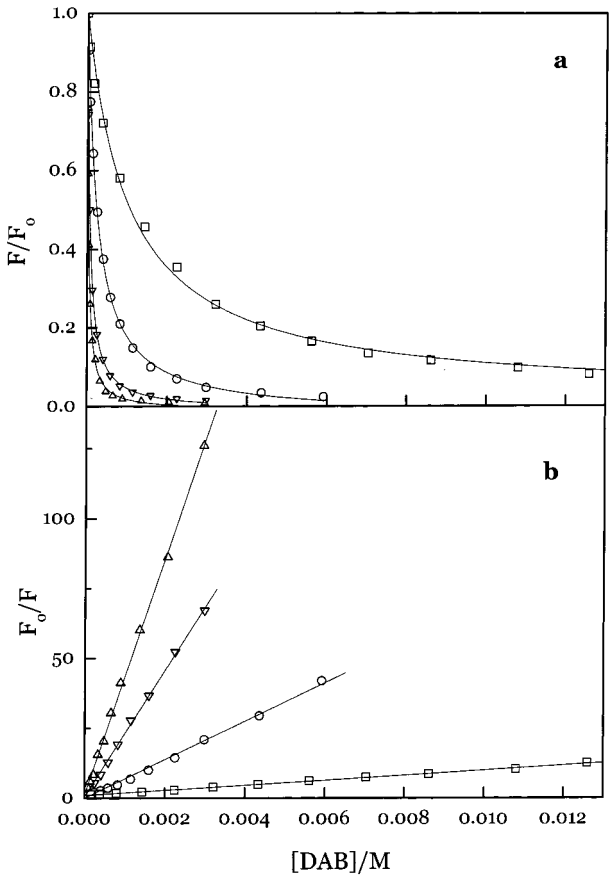

Figure 1. (a) Plots of F/F_o vs dendrimer concentration, [pyrene] $= 6.7 \times 10^{-7}$ M. Continuous lines represent computer fits of eq 5 to the experimental points, from which the K_b values (Table 1) were obtained. (b) Stern−Volmer plots from which the quenching constants k_q of Table 1 were obtained. Symbols: (□) DAB-4, (○) DAB-16, (▽) DAB-32, and (△) DAB-64.

Table 1. Binding (K_b) and Quenching (k_q) Constants of the Interaction between Pyrene and DAB Dendrimers[a]

dendrimer	F_b/F_o	K_b (M^{-1})	R^2	k_q ($M^{-1}s^{-1}$)	R^2
DAB-4	0.002	898	0.9994	6.2×10^9	0.9994
DAB-16	0.001	3674	0.9999	4.7×10^{10}	0.9991
DAB-32	0.002	16645	0.9999	1.6×10^{11}	0.9997
DAB-64	0.003	32930	0.9999	2.9×10^{11}	0.9999

[a] F_b/F_o is the ratio in the fitting eq 5. The first R^2 refers to the goodness of the fit of eq 5 and the second to that of eq 6. The fluorescence lifetime of pyrene in nondegassed aqueous solution is $\tau = 144$ ns.

of pyrene, f_b and f_f,

$$F = f_f F_o + f_b F_b \quad (4)$$

and the experimentally accessible parameters F_o and F_b, the latter representing the fluorescence intensity at the completion of the titration with the aqueous dendrimer solution. Finally, from eqs 3 and 4 we obtained eq 5, which is the appropriate fitting equation. When eq 5 was

$$F/F_o = 1 + ((F_b/F_o) - 1)K_b[\text{DAB}]/(1 + K_b[\text{DAB}]) \quad (5)$$

fitted to the experimental data, viz., F/F_o vs [DAB], K_b and the ratio F_b/F_o were obtained. Such fittings, along with the experimental data, are shown in Figure 1a, from which the values for the binding constants K_b, shown in Table 1, were obtained, whereas the ratio F_b/F_o turned out to be very close to zero in all cases. The increased

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values of K_b at higher dendrimer generations, reflect the increased solubilization of pyrene when the size of the dendrimer is increased. This is also confirmed by the intensity ratio I_1/I_3 of the first to the third fluorescence peaks of pyrene, which is an established index for the polarity of the environment of pyrene.[16] Thus, in DAB-4 this ratio remains constant throughout the titration with the dendrimer solution, its value being ca. 1.6 which corresponds to aqueous polarity; therefore, pyrene, when solubilized in DAB-4, resides close to the water phase at all dendrimer concentrations. By contrast, in higher dendrimer generations the I_1/I_3 ratio decreases with increasing [DAB] approaching the value corresponding to hexane ($I_1/I_3 = 0.6$) at the highest DAB-64 concentration. We conclude therefore that as the dendrimer generation increases pyrene is pushed deeper and deeper inside the dendrimer structure where it is presumably well protected from the water phase.

Pyrene Fluorescence Quenching. Since from the computer fittings (Figure 1a) it was found that the F_b/F_o ratio is always very close to zero (see Table 1), we can eliminate it from eq 5, which then, upon inversion, takes the form of the well-known Stern−Volmer relationship (eq 6),

$$F_o/F = 1 + K_b[\mathrm{DAB}] = 1 + k_q[\mathrm{DAB}] \qquad (6)$$

where [DAB] stands for the total concentration of the quencher added at any moment. Evidently, the binding constant K_b is equal to the slope of the Stern−Volmer straight line, which is equal to the product of the quenching constant k_q times the fluorescence lifetime τ of pyrene in water. Stern−Volmer plots according to eq 6 are shown in Figure 1b, whereas the extracted magnitudes of k_q are listed in Table 1. The large quenching constants k_q, shown in Table 1, by far exceed the values expected for diffusion controlled quenching. Indeed, the k_q values measured here with the dendrimer quenchers are at least 10−100 times larger than k_q estimated from the Smoluchowski equation. These results may be rationalized in terms of the very high effective concentration of quenching centers (tertiary N) inside the dendrimers, where most of the pyrene fluorophore resides. Note also that the large k_q values are in good agreement with the equally large binding constants K_b. Moreover, the magnitudes of both of these parameters, k_q and K_b, are linearly dependent on the dendrimer molecular weight, as shown in Figure 2. Recall that similar behavior has been observed in other dendrimers when pyrene was used as guest molecule in dendritic hosts.[17] It is also interesting to note that when DAB-4 is used as the quencher of the fluorescence of pyrene, the magnitude of k_q is more than twice as much in water than it is in ethanol ($k_{qwater} = 6 \times 10^9\ M^{-1}\ s^{-1}$; $k_{qethanol} = 2.5 \times 10^9\ M^{-1}\ s^{-1}$), although the viscosity of the later is only 20% higher than of the former ($\tau_{water} = 1$; $\tau_{ethanol} = 1.2$ cP). Similar results, i.e., lower quenching constants than in water, were obtained with other solvents, viz., toluene and chloroform, and with all other generations of DAB dendrimers. Furthermore, even when triethanolamine, which forms homogeneous solutions in both water and ethanol and which also possess a tertiary N, $N(CH_2CH_2OH)_3$, was used as quencher instead of DAB, it was found that k_q was equal to 5.5×10^8 in ethanol, while it was as much as 4 times more in water, $k_q = 2 \times 10^9$. These results indicate that some specific interaction occurs in water between pyrene and the tertiary amino group. In addition, dilute aqueous solutions of $(6-7) \times 10^{-7}$ M pyrene in water exhibited a broad fluorescence peak around 480 nm when DAB-4 was added to the solution (note that pyrene alone in water does not exhibit any emission at this wavelength). The intensity of this emission decreased with increasing dendrimer generation. Interestingly, this broad pyrene fluorescence (at 480 nm) which appears when DAB-4 is added to pyrene solutions, occurs only in water and not in other solvents, either polar e.g. ethanol, or nonpolar, e.g., toluene. To recapitulate, the monomeric fluorescence of pyrene undergoes very effective quenching by tertiary N in water, with the simultaneous appearance of a broad emission at ca. 480 nm. In other solvents however, except water, such broad emission was not observed. This kind of quenching of the fluorescence of pyrene by tertiary N, namely, with the simultaneous appearance of the novel broad emission at 480 nm (which occurs only in water), was observed in both homogeneous and dendritic solutions. To further investigate this behavior we studied aqueous dendrimer solutions containing increased amounts of pyrene.

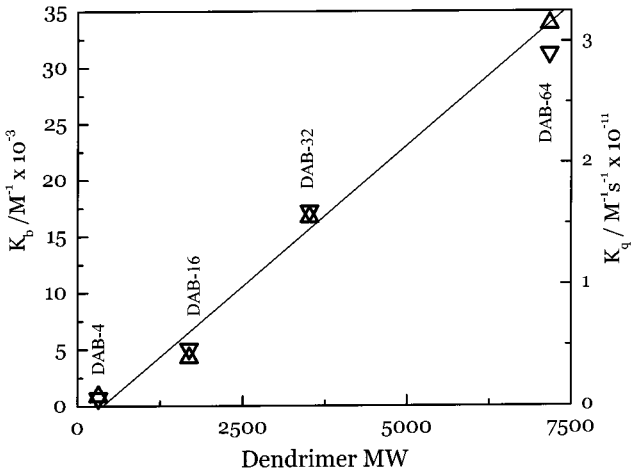

Figure 2. Plot of binding constant K_b (△) and quenching constant k_q (▽) vs dendrimer molecular weight.

Emissive Exciplex Formation. The fluorescence spectra of aqueous solutions containing constant pyrene concentration and equal masses of DAB, viz., [Py] = 4.5×10^{-6} M, [DAB] = 8.24 g/L, exhibit broad fluorescence around 480 nm, the intensity of which decreases progressively with increasing dendrimer generation, from DAB-4 to DAB-64, as shown in Figure 3. We then examined the dependence of the intensity of the broad emission on the concentration of pyrene, at constant dendrimer mass, as shown in Figure 4. It was found that as pyrene was added to aqueous solutions of DAB the intensity of the emission at 480 nm increased at a rate which was much faster in DAB-4 than in DAB-64. Although the fluorescence at 480 nm resembles very mush the excimer fluorescence of pyrene, this emission cannot be excimeric, since according to the Poisson statistics under the present conditions ([DAB] = $1.9 \times 10^{-2}-1.15 \times 10^{-3}$ M, [Py] < 2.5×10^{-5} M) the probability of a dendrimer unit being occupied by two pyrene molecules is negligible (ca. 2×10^{-4}). On the other hand, it has been reported in the literature that tertiary amines quench the fluorescence of pyrene in nonaqueous media, via exciplex formation with the simultaneous appearance of some extremely weak broad emission at longer wavelengths.[6-9] In such exciplexes pyrene is the electron acceptor and the tertiary amine group is the donor. We have therefore assigned the emission at 480 nm to the fluorescence of exciplexes formed between pyrene and the

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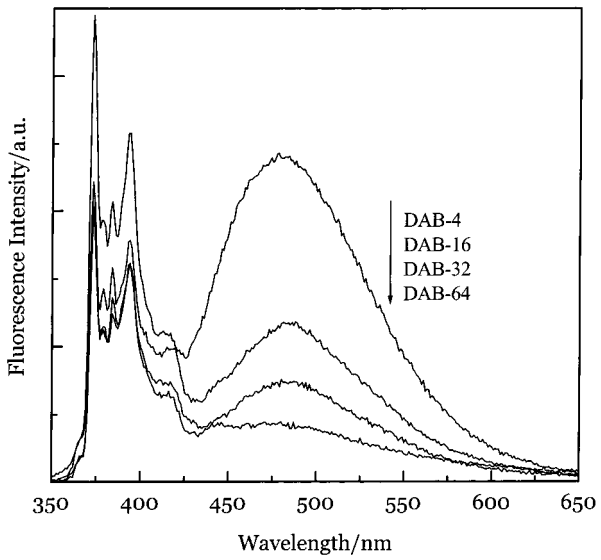

Figure 3. Fluorescence spectra of aqueous solutions of pyrene and equal masses of [DAB], viz., [pyrene] = 4.5×10^{-6} M and [DAB-4] = [DAB-16] = [DAB-32] = [DAB-64] = 8.24 g/L. Note that because of the low solubilization of pyrene in DAB-4, in this aqueous dendrimer solution pyrene concentration is not 4.5×10^{-6} M but 2.33×10^{-6} M. Excitation wavelength 335 nm.

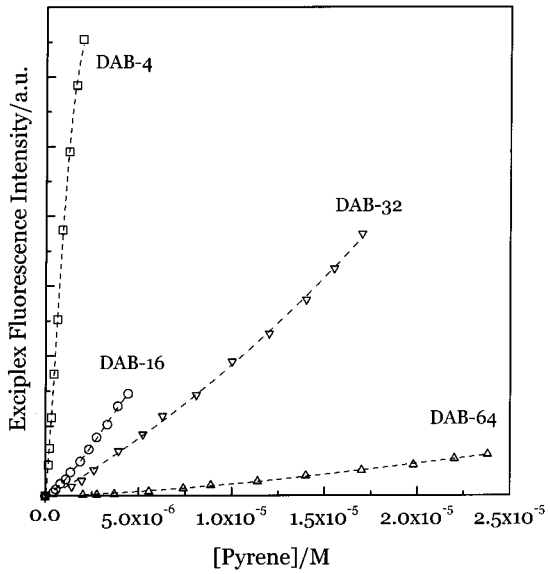

Figure 4. Plots of the intensity of the broad emission (480 nm) in arbitrary units (a.u) vs pyrene concentration. The concentrations of the aqueous dendrimer solutions were chosen to contain equal DAB masses, viz., 8.24 g/L; thus, [DAB-4] = 1.84×10^{-2} M = 4[DAB-16] = 8[DAB-32] = 16[DAB-64]. Symbols are the same as those in Figure 1. Note that the increase in the maximum pyrene concentration taken up by the dendrimers (viz., DAB-4[Py] $\cong 2.5 \times 10^{-6}$ M; DAB-64[Py] $\cong 2.4 \times 10^{-5}$ M) reflects differences in the corresponding binding constants, see Table 1. Dotted lines do not correspond to computer fits.

tertiary nitrogens at the branching points of the dendrimer. There are however several differences, to be discussed presently, between ordinary exciplexes and the ones observed here.

Of particular interest are the excitation spectra of pyrene solubilized in aqueous solutions of DAB. These spectra are different, depending on whether the fluorescence is monitored at 373 nm (i.e. the maximum of pyrene's monomeric emission, see Figure 3) or at 480 nm (i.e. the maximum of the exciplex emission). Indeed, Figure 5a shows that the two excitation spectra are shifted by ca.

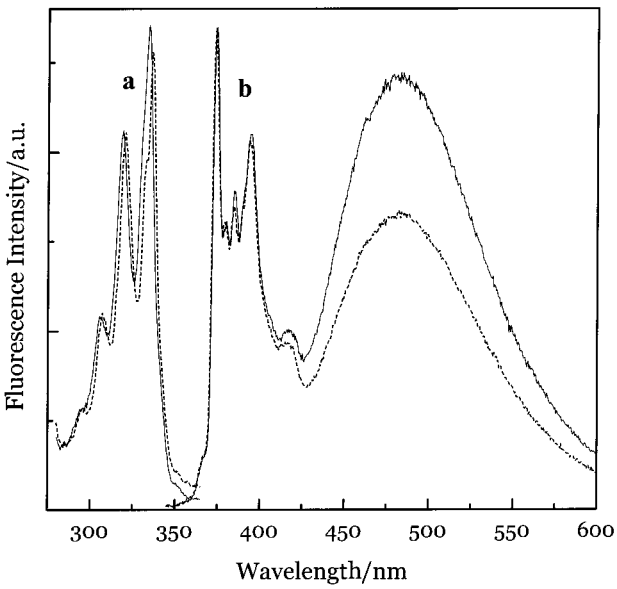

Figure 5. Aqueous solution of 1.77×10^{-5} M pyrene and 2.3×10^{-3} M DAB-32. (a) Excitation spectra: solid line, emission monitored at the maximum of the monomer fluorescence (373 nm); dotted line, emission monitored at the maximum of the broad fluorescence (480 nm). (b) Fluorescence spectra: solid line, obtained by exciting at the maximum of the excitation spectrum of the monomeric pyrene (337 nm); dotted line, obtained by exciting at the maximum of the excitation spectrum of the pyrene dimmer (335 nm).

2 nm with respect to each other, the first peak of the excitation spectrum occurring at 335 nm when the emission is monitored at the monomer fluorescence (373 nm) and at 337 nm when the emission is monitored at the exciplex emission (480 nm). This indicates that there are two different kinds of pyrene molecules in aqueous DAB solutions, one molecule (say, A) which produces the excitation spectrum having the excitation peak at 335 nm and another (say, B) which produces the red-shifted excitation spectrum having the peak at 337 nm. Furthermore, Figure 5b shows that different fluorescence spectra are obtained when exciting at 335 nm (i.e., exciting pyrene molecules A), than when exciting at 337 nm (i.e., exciting pyrene molecules B). All these findings constitute convincing evidence that there is ground state interaction involving pyrene molecules B, which results in the modification of the energy levels of pyrene with respect to the free, noninteracting, pyrene molecules A. Presumably this interaction takes place between pyrene and a tertiary nitrogen at the branching points of the dendrimer. We must point out however that ground state interaction, like the one we have observed here, does not ordinarily occur in exciplexes, although exceptions have been reported, e.g., between *N*,*N*-dimethylaniline and pyrene.[18] At this point it should also be emphasized that this unusual formation of emissive exciplexes is not restricted to dendrimers but it is also observed in homogeneous solutions. Thus Figure 6 shows excitation and fluorescence spectra of pyrene quenched by $N(CH_2CH_2OH)_3$ in water. Two different excitation spectra (inset Figure 6) were obtained depending on the wavelength at which the emission was monitored, and also two different fluorescence spectra were obtained depending on the excitation wavelength. When however the solvent was other than water, e.g., ethanol, toluene, or chloroform, the quenching of the fluorescence of pyrene by tertiary amine groups,

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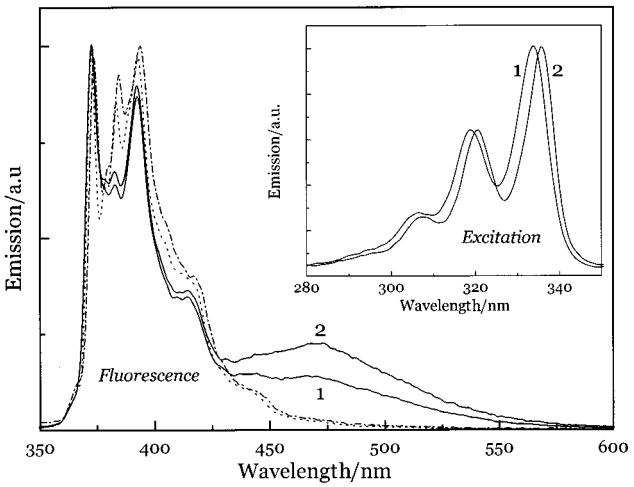

Figure 6. Fluorescence spectra of 7×10^{-7} M pyrene, partially quenched by 1.88×10^{-2} M of $N(CH_2CH_2OH)_3$, in toluene (dotted line), chloroform (dashed−dotted line), and water (solid lines, 1 excited at 334 nm, 2 excited at 336 nm). Inset: Excitation spectra of the same aqueous solutions, 1 fluorescence monitored at 373 nm, 2 at 480 nm. In the other solvents the excitation spectra were wavelength independent.

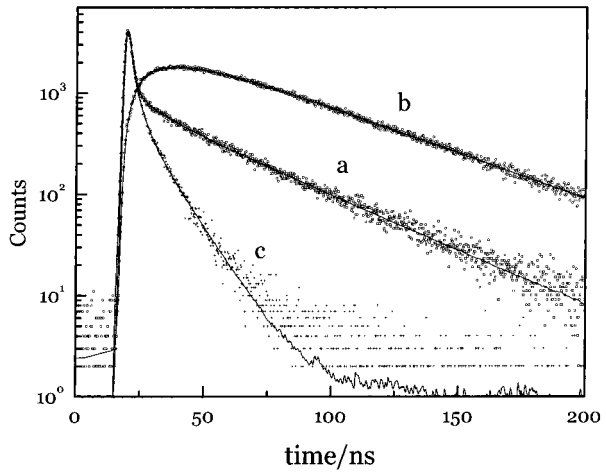

Figure 7. Fluorescence decay profiles at 373 nm (monomer emission) and at 480 nm (exciplex emission), [pyrene] = 1.76×10^{-5} M dissolved in [DAB-32] = 2.3×10^{-3} M aqueous solutions, before and after the addition of [triethanolamine] = 5×10^{-2} M. (a) Decay at 373 nm (monomer pyrene) before addition of trietahnolamine. (b) Decay at 480 nm (exciplex), before and after addition of triethanolamine. (c) Monomer decay after addition of triethanolamine;

either in homogeneous or in dendritic media, did not involve emissive exciplex formation (Figure 6).

To further investigate the exciplex formation in these dendrimers we studied the time-resolved monomer and exciplex fluorescence decays of pyrene in aqueous DAB solutions, before and after the addition of the hydrophilic fluorescence quencher triethanolamine. Figure 7 shows the fluorescence decay profiles of a typical sample containing 4.5×10^{-6} M pyrene and 2.3×10^{-3} M DAB-32 in water. We found that, before the addition of triethanolamine, three different decays make up the overall fluorescence decay of the monomer (decay at 373 nm), viz., $\tau_1 \cong 1-2$ ns, $\tau_2 \cong 8-11$ ns, and $\tau_3 \cong 30-50$ ns, while the analysis of the exciplex emission (decay at 480 nm), produced a rise time (negative preexponential factor), $\tau_4 \cong 9-12$ ns, and one decay lifetime, $\tau_5 \cong 45-50$ ns. Note that the fluorescence lifetime of pyrene dissolved in pure water, which in our nondegassed solutions is equal to 144 ns, is absent from the decays of Figure 7. We have

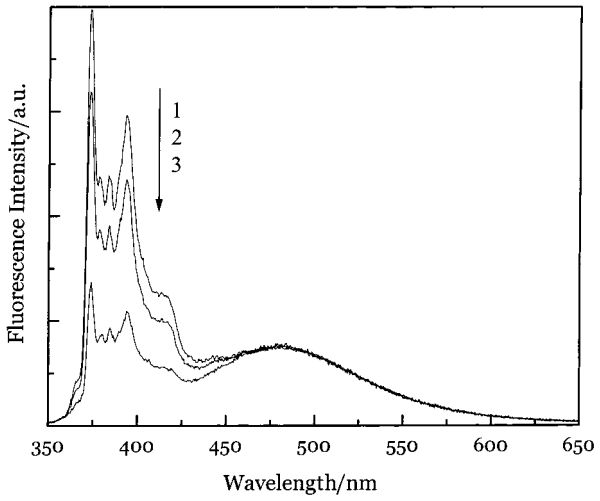

Figure 8. Quenching of the fluorescence of 5.73×10^{-6} M pyrene in 4.6×10^{-3} M aqueous solution of DAB-16. Quencher triethanolamine 1. 0 M, 2. 10^{-2} M, 3. 2×10^{-1} M. Note the decrease of the ratio I_1/I_3 from 1.68 in 1 to 1.46 in 3 with the addition of the quencher. (See text.)

interpreted this as indication that all the free pyrene molecules, i.e., molecules not permanently bound to the dendrimer, are rapidly exchanged between the aqueous and the dendritic phases. To these exchanging molecules we have assigned the $\tau_3 \cong 30-50$ ns fluorescence lifetime, an assignment which was further confirmed by the next experiment. Indeed, we found that upon addition of the strongly hydrophilic triethanolamine, which is a good quencher of the fluorescence of pyrene, τ_3 disappeared from the decay profile, but τ_1, τ_2, τ_4, and τ_5 were not at all affected. These results constitute convincing evidence that τ_3 is the lifetime of water accessible pyrene, while all the other pyrene molecules, to which the decay parameters τ_1, τ_2, τ_4, and τ_5 correspond, reside inside the dendrimer where they are well protected from hydrophilic molecules. Furthermore, the fact that $\tau_4 \cong 9-12$ ns, the rise time of the broad emission, coincides with $\tau_2 \cong 8-11$ ns, the time parameter of the monomer decay, leads to the conclusion that τ_2 is the time interval during which one excited pyrene molecule rearranges in order to interact and form the species emitting the broad fluorescence with lifetime $\tau_5 \cong 45-50$ ns. Note also that $\tau_2 \cong 8-11$ ns does not appear in the time-resolved fluorescence decays from solutions which do not exhibit the broad emission, i.e., solutions containing minute pyrene concentrations. This further confirms that τ_2 is directly associated with the species emitting at 480 nm. Finally, the shortest time, $\tau_1 \cong 1-2$ ns, we have assigned to pyrene molecules which although they are solubilized inside the dendrimer they do not interact, but instead are rapidly quenched by the very high effective concentration (estimated, ca. 30−50 M) of the tertiary nitrogen quenchers.

Steady-state fluorescence spectra corroborated the above results, thus, when to 5.7×10^{-6} M pyrene in 4.6×10^{-3} M aqueous DAB-16 solution, increasing amounts of the fluorescence quencher triethanolamine were added, the intensity of the broad fluorescence at 480 nm was remained practically unaffected, while that of the monomer drastically decreased (see Figure 8). Also, the ratio I_1/I_3 of the monomer fluorescence changed with the addition of triethanolamine, from 1.68 (corresponding to the polarity of water) to 1.46 (corresponding to a considerably less polar environment). These findings indicate that the unquenched pyrene molecules—both those emitting the residual monomeric and those emitting the

exciplex fluorescence—reside in a less hydrophilic region (viz. inside the dendrimers) than the ones quenched by triethanolamine, which reside mostly in the bulk water and therefore are readily accessible to the hydrophilic quencher.

In conclusion, the following results were obtained from this study: (i) Solubilization of pyrene in DAB dendrimers increases linearly with increasing dendrimer generation. (ii) It was found that the well-known quenching of the fluorescence of pyrene by the tertiary amine group, which occurs via exciplex formation, involves emissive exciplexes only in aqueous environment, in all other solvents studied here the quenching was not followed by any additional emission. (iii) Making use of this finding concerning exciplex formation, we were able to show conclusively that as the DAB generation increases water is progressively excluded from the dendritic interior.

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