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pH-Dependent Encapsulation of Pyrene in PPI-Core:PAMAM-Shell Dendrimers

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Core−shell dendrimers consisting of poly(propyleneimine) (PPI) dendrimer as a core and poly(amidoamine) (PAMAM) dendrons as a shell have been synthesized through the route of Michael addition reaction followed by amidation. These macromolecules were investigated their ability to solubilize a guest molecule, pyrene. The number of encapsulated pyrene molecules per dendrimer increased with pH of a solution and generation (G) of PAMAM dendron, and it reached 2.7 for PPI(G3)−core:PAMAM(G3)-shell dendrimer at pH 11. It was confirmed that the solubilized pyrene located in the hydrophobic nanocavities of the PPI dendrimer core in the dendrimer. The shrunk PAMAM dendron shell should play a role of retention fence of doped molecules.

Introduction

Dendrimers are a highly diverse, unique class of polymers that are a well-defined macromolecular architecture, unlike traditional polymers.[1,2] They are constructed by the iteration of branching unit growth, called generation (G), from a multifunctional core. Thus, dendrimers appear to be unimolecular micelles[3−6] owing to their similarity in size and structure to surfactant micelles. Then surfactant micelles are formed only at a concentration above the critical micelle concentration, vary significantly in size and shape, and are thermodynamically equilibrium systems, in which surfactant monomers continuously migrate into and out of the micelle. However, dendrimers do not share these properties. Instead, they are covalently bound monodisperse molecules,[7] easily functionalized,[8,9] and stable over wide pH and concentration ranges,[10] if they are adequately controlled their solubility and stability in many organic solvents. Therefore, the substitution of dendrimers for surfactant micelles should be particularly valuable in applications, for example, as drug delivery systems, gene transfer agents,[11,12] reaction reservoirs,[13,14] and molecular recognition systems.[15] Then the applications of dendrimers will diversify as well as their structures.

In light of drug delivery systems involving dendrimers, we now examine a model system based on G3 poly(propyleneimine) (PPI) dendrimer as a hydrophobic core and poly(amidoamine) (PAMAM) branches as a hydrophilic shell. This type of core−shell dendrimers has been synthesized and characterized by Majoros et al.[16] Incorporation of the functional drug molecule in the dendrimer and its controlled release on exposure to different environment are prerequisites for effective drug delivery systems. In consideration of this issue, the present investigation was focused on whether changing the pH environment of the dendrimer solutions affects the solubilization ability of dendrimers for guest molecules like pyrene or not. The PPI-core:PAMAM-shell dendrimers possess tertiary amine repeating units and are therefore susceptible to protonation, which will certainly change the environment of the nanocavities in the dendrimer, bringing in turn the either favoring or nonfavoring solubilization of guests depending on the polarity of the guests used.

Experimental Section

Materials. PPI dendrimer (G3), ethylenediamine, methyl acrylate, and methanol were purchased from Aldrich and used without further purification. Commercial pyrene and ethanol (analytical reagent grade) were used without further purification. Water was purified by distillation and deionization using a Millipore Milli-Q Laboratory purification system.

Propagation of PAMAM dendrons on PPI dendrimer was followed by a conventional divergent procedure.[15,16] That is, PPI-core:PAMAM-shell dendrimers were synthesized from PPI core by following two steps, namely, Michael addition and amidation reactions. Excess reagents and solvents were removed completely, and the products were purified prior to characterization. A typical synthetic procedure is as follows: amine-terminal G3 PPI dendrimer (0.1 g) was dissolved in 50 cm^3 of methanol, and a methanol solution of methyl acrylate (0.162 mg, 1.89 mmol) was added dropwise. The mixture was stirred for 2 days at room temperature. The solvent and excess methyl acrylate were vacuum-distilled to leave over pale yellow product, PPI(G3)−PAMAM(G0.5) dendrimer. FW: 4441.2. IR: 3342, 2942, 2856, 1735, 1641, 1549, 1433, 1356, 1327, 1274, 1030 cm^{-1}.

The amidation reaction was performed as follows: Half generation product (0.1 g, 0.022 mmol) was dissolved in 30 cm^3 of

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methanol, and a methanol solution of ethylenediamine (4.2 g, 0.07 mol) was added dropwise. The mixture was stirred for 4 days at room temperature. The solvent and excess methyl acrylate were vacuum-distilled to result in viscous, yellow oily product. Michael addition and amidation reaction procedures were successively carried out to synthesize a PPI(G3)−PAMAM (G3) dendrimer. PPI(G3)−PAMAM(G1) dendrimer: IR: 3345, 3185, 2854, 1641, 1549, 1433, 1356, 1327, 1274, 1030 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 0.96−0.98 (m, 56H), 1.3−1.38 (m, 112H), 2.55−2.60 (m, 48H), 2.0−2.08 (s, 64H), 2.91−2.98 (m, 64H), 3.22−3.46 (m, 32H). PPI(G3)−PAMAM(G2) dendrimer: IR: 3345, 3185, 2854, 1641, 1549, 1433, 1356, 1327, 1274, 1030 cm^{-1}. ^1H NMR (500 MHz, D_2O, δ): 1.96−1.99 (m, 32H), 2.0−2.07 (m, 64H), 2.36−2.39 (m, 64H), 2.64−2.70 (m, 88H), 2.91−3.98 (m, 64H), 3.0−3.08 (m, 64H), 8.0−8.10 (m, 128H). ^{13}C NMR = 29.4, 34.0, 42.8, 43.8, 47.4, 48.6, 49.1, 50.5, 176.1 (C═O). PPI(G3)−PAMAM(G3) dendrimer: IR: 3345, 3185, 2854, 1641, 1549, 1433, 1356, 1327, 1274, 1030 cm^{-1}. ^1H NMR (500 MHz, D_2O, δ): 2.18−2.10 (m, 16H), 2.28−2.50 (m, 152H), 2.91−3.31 (m, 128H), 3.46−3.86 (m, 152H), 7.9−8.2 (m, 224H). ^{13}C NMR = 32.0, 42.8, 43.7, 47.2, 48.9, 49.3, 49.6, 52.2, 54.9, 175.9 (C═O). Hydrodynamic diameter: 7.9 nm (see Supporting Information).

Measurements. The solubilization of pyrene was examined as follows: Excess solids of pyrene were dispersed in an aqueous solution of PPI-core:PAMAM-shell dendrimer at 1.0 mg cm^{-3}, and the dispersion was stirred for 24 h. Insoluble solids were removed by filtration (Millipore filter, 0.45 μm pore size). Then ethanol was added into the filtrate. The same procedure was carried out for pure water without dendrimer. Separately, aqueous ethanol solutions of dendrimers at 1.0 mg cm^{-3} were also prepared.

UV−vis absorption spectroscopic measurements were performed using a Shimazdu UV-2200 instrument, which was temperature-regulated at 25 °C during the measurement. Absorbance at a UV−vis absorption band in a pyrene-solubilized PPI−PAMAM dendrimer solution was subtracted from those of a pyrene-nonsolubilized PPI−PAMAM dendrimer solution and a pyrene-solubilized water at corresponding ethanol volume %. Uptake was evaluated as the number of pyrene in a dendrimer.

On a typical run of titration experiments, an aqueous dendrimer solution was progressively added to pyrene-containing water, and the fluorescence of pyrene was monitored to evaluate the ratio, I_3/I_1. Fluorescence spectra were recorded at 25 °C under excitation at 334 nm on a Hitachi F-4010 fluorometer.

Fourier transform-infrared (FT-IR) absorption spectra were recorded using a Bio-Rad FTS 575C spectrometer equipped with a cryogenic mercury cadmium telluride detector. A drop of dendrimer was sandwiched between two KBr pellets to record FTIR spectra. Nuclear magnetic resonance (NMR) spectra were recorded for deuterated aqueous solutions (1 mg cm^{-3}) of dendrimers on a JEOL JNM-L500. All measurements were carried out at room temperature. Dynamic light scattering (DLS) was measured using a Zetasizer Nanoseries instrument at 25 °C. An aqueous dendrimer (10^{-3} M) solution has been used to study DLS, and the solutions were filtered through 0.1 μm filters. A hydrodynamic diameter was obtained.

Results and Discussion

Synthesis of PAMAM dendron shell opening with G3 PPI dendrimer core started from Michael addition reaction of methyl acrylate with amine terminals of PPI dendrimer. In this reaction, a slight excess (10%) of methyl acrylate was used. However, on the amidation process, a very large excess of ethylenediamine (20 equiv) was needed to prevent bridging and gelation of dendrimers. Excess reagents and solvents were removed under vacuum at a temperature below 50 °C.

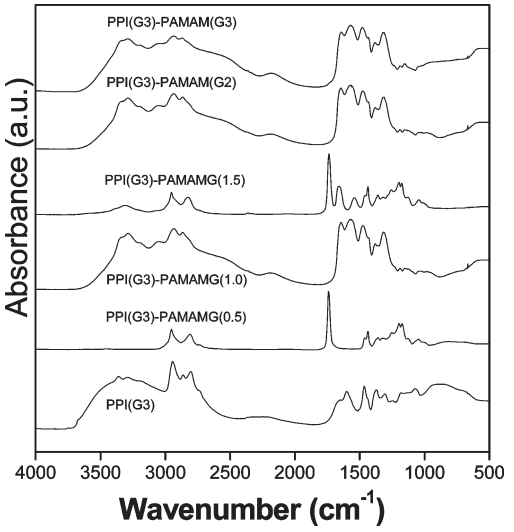

Figure 1. IR spectra of PPI-core:PAMAM-shell dendrimers with different generations of PAMAM dendron.

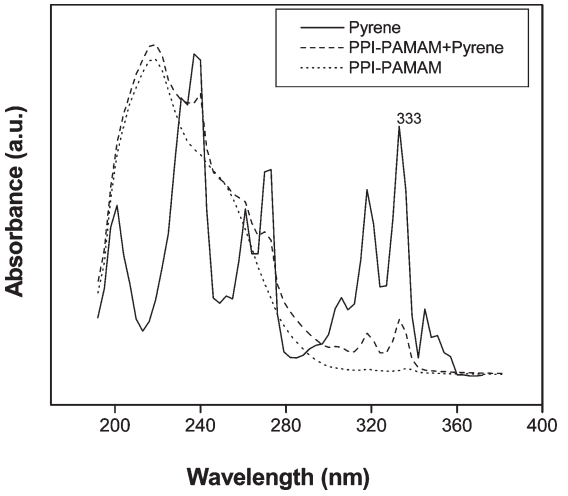

Figure 2. UV−vis absorption spectra of PPI(G3)−PAMAM(G3) dendrimer, pyrene, and pyrene-solubilized PPI(G3)−PAMAM (G3) dendrimer.

The reaction progress of Michael addition and amidation was monitored by using IR absorption spectroscopy. Comparative IR spectra of PPI(G3) with 16 terminals, half-generation PPI(G3)−PAMAM(G0.5, 1.5) with 32 and 64 terminals, respectively, and full generation PPI(G3)−PAMAM(G1, 2, 3) dendrimers with 32, 64, and 128 terminals, respectively, are shown in Figure 1. Half-generation dendrimers reveal an ester (C═O) stretching band at 1735 cm^{-1}. However, PPI(G3) and full generation dendrimers display bands at 1593 and 1477 cm^{-1} for NH_2 deformation modes, confirming the presence of amino terminals.[17] The IR spectra of half generation PPI(G3)−PAMAM(G1.5) and all full generation dendrimers also show bands at 1670 and 1541 cm^{-1} for amide I and II modes, respectively, of amide bonds, although the latter band is superimposed on a NH_2 deformation band. These results indicate the successful synthesis of PPI-core:PAMAM-shell dendrimers.

The UV−vis absorption spectra of pyrene and pyrene-solubilized dendrimer are presented in Figure 2. The characteristic absorption band of pyrene was observed at 333 nm, which was also obtained for pyrene-solubilized dendrimer but not for pris-

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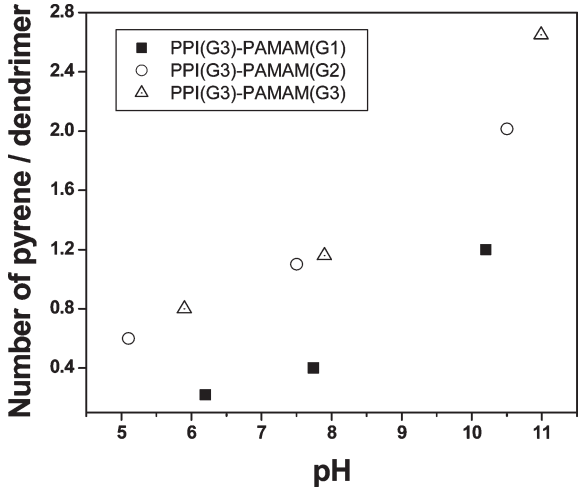

Figure 3. pH dependence of pyrene solubility in PPI-core:PAMAM-shell dendrimers.

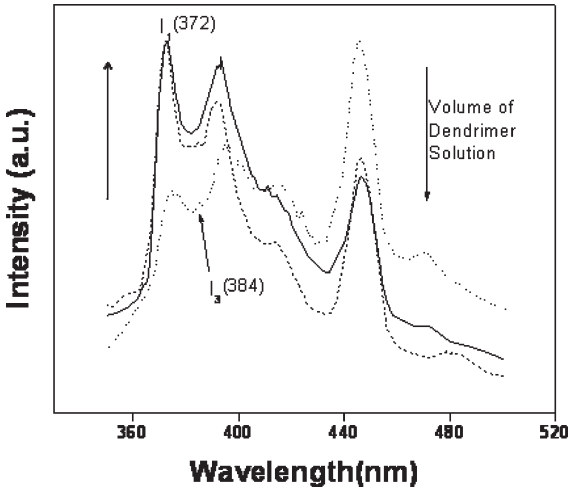

Figure 4. Variation of pyrene fluorescence in water with adding dendrimer.

tine dendrimer. Therefore, this absorption band was used for calculating the number of pyrene solubilized in dendrimer, as described in the Experimental Section. The pH dependence of pyrene solubility, evaluated from absorption spectroscopy, is plotted in Figure 3. It can be confirmed that larger number of pyrene molecules is solubilized in dendrimer at basic pH than at acidic pH. The solubilization attained 2.7 pyrene molecules per PPI(G3)−PAMAM(G3) dendrimer at pH 11. It should be also noted that the solubility of pyrene in the dendrimers is dependent on the size of the PAMAM dendrimer between G1 and G2 but independent of the size of G2 and G3.

The pK_a values of primary and tertiary amines are respectively 9.75 and 6.1 for PPI dendrimer and 9.0−9.3 and 6.0−7.3 for PAMAM denderimer.[18−21] Then at pH above 10, almost of the amino groups in dendrimer are scarcely protonated in water. As the pH value of the solution is lowered down to below 9 by adding hydrochloric acid, peripheral primary amino groups are fairly protonated. When the pH value is further reduced, the tertiary amino groups in the interior of the dendrimer undergo protonation, which is considerably completed at about pH 5. Finally, the amines in the core and the periphery achieve their protonation at lower pH values. Then the degree of protonation should concern to the solubility of pyrene.

In a progressive addition of dendrimer to an aqueous solution containing pyrene of 6.7×10^{-7} M (a concentration lower than the solubility, 8×10^{-7} M, of this fluorophore in water), strong quenching of fluorescence intensity of pyrene was observed, as shown in Figure 4. Meanwhile, when ethanol was used as a solvent instead of water, fluorescence quenching was not observed, since pyrene can preferably dissolve in the bulk ethanol phase and does not need to enter into the dendrimer nanocavities. The decrease of fluorescence intensity of a 446 nm band, as seen in Figure 4, must therefore be attributed to the binding of pyrene to dendrimer. This conclusion is also supported by the following observation: When ethylenediamine, consisting of the terminal NH_2 functional groups similar to dendrimers, was added to an aqueous solution of pyrene, the fluorescence was not quenched. In contrast, triethanolamine, which structurally resembles the

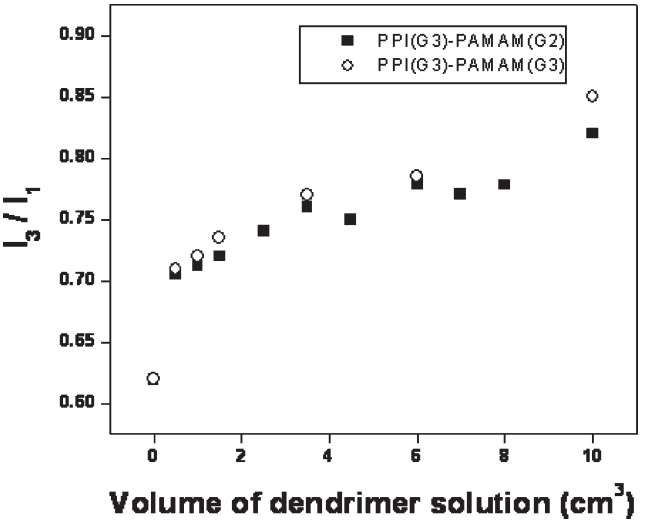

Figure 5. Plot of I_3/I_1 of pyrene as a function of added volume of a dendrimer solution.

branching moiety (the tertiary amine) of the present dendrimers, caused significant quenching of pyrene fluorescence in water owing to the electron transfer from triethanolamine to photoexcited pyrene.[22]

Further, the location of pyrene could be more strictly confirmed by plotting I_3/I_1 (third/first vibronic fluorescence band intensity) ratio against volume of dendrimer added to a pyrene solution (Figure 5), since the I_3/I_1 ratio is very sensitive to the nanoenvironment around pyrene.[23] The I_3/I_1 ratio increased from 0.7 to 0.85 with increasing dendrimer volume, although it was almost independent of PAMAM dendrimer generation of G2 and G3. The increase in I_3/I_1 ratio suggests that the pyrene is surrounded by hydrophobic environment. Then the present observations confirm that the solubilized pyrene molecules reside inside the dendrimer nanocavities close to the tertiary amino groups, which are evidently responsible for fluorescence behavior.

Although the doping or solubilization of fluorescence-probing hydrophobic guest molecule, pyrene, in dendritic polymers has

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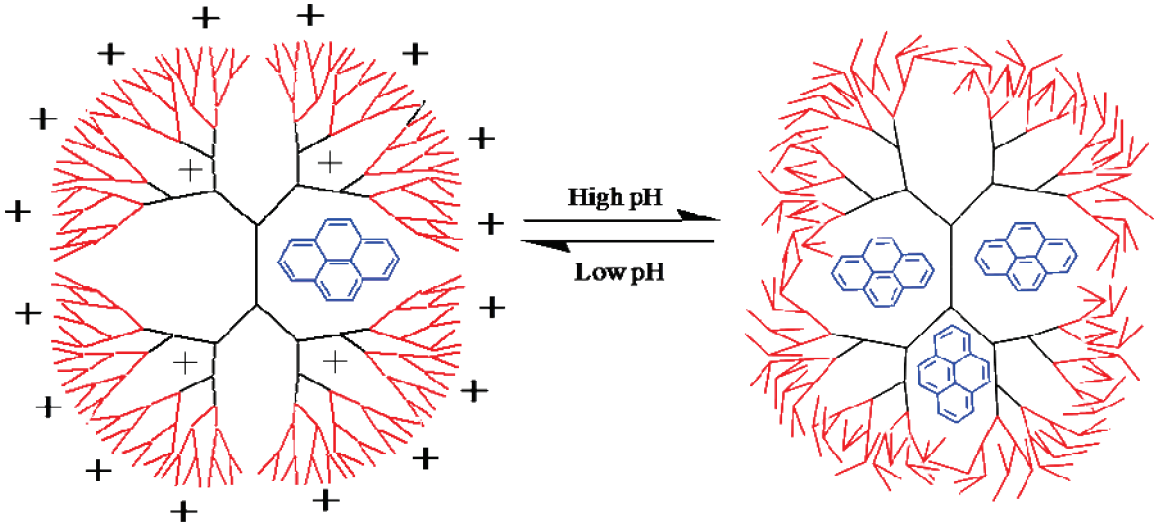

Figure 6. Schematic representation of pyrene encapsulation in PPI-core:PAMAM-shell dendrimers at acidic and basic pH.

been investigated some groups,[24−31] the quantitative reports concerning the number of solubilized pyrene are few. Apart from polymer micelles which consist of block copolymers with hydrophobic dendron pendant chains and have large nanocavities, doping 23 pyrene molecules in a dendron block core,[30] conventional or amphiphilic dendrimers can dope only one or less pyrene per dendrimer.[27,31] However, the detailed examination in our previous report confirms that the solubility of pyrene depends on the size of nanocavities in PAMAM and PPI dendrimers which are controlled by terminal groups, internal chemical structures, generations, and protonation of dendrimers.[29] It should be noticed that amine-terminated G4 PPI dendrimers doped only 0.4, 0.6, and 0 pyrene molecules at pH 5.9, 8.5, and 11.0, respectively.[29] Thus, it can be mentioned from the preset work that the core−shell structures give rise to better pyrene solubilization than that of simple PPI or PAMAM dendrimers.

The polarity index, that is, I_3/I_1 value, should also be compared. The I_3/I_1 value of pyrene-doped core−shell dendrimers was higher than that (0.5−0.6: close to that in water and independent of pH) of simple PPI or PAMAM dendrimers,[29] indicating pyrene located in hydrophobic environment of the core−shell dendrimer. Then the data in the present work allows some insight concerning the solubilization site for pyrene molecules in the core−shell dendrimers. Namely, pyrene probe molecules are encapsulated in the inner PPI dendrimer core, which are protected from water penetration by hydrophilic PAMAM dendron shell to be hydrophobic. The PAMAM dendron shell plays a role of keeping PPI dendrimer core structure not to be shrunk and of retaining dopants in the core. Especially, the latter role is more effective at high pH because of the shrunk structure of nonprotonated PAMAM dendrons (see Figure 6).

Conclusions

In aqueous media, PPI-core:PAMAM-shell dendrimers gave full play to their solubilization ability toward hydrophobic guest molecules as compared to homodendrimers such as PPI and PAMAM dendrimers: Pyrene was solubilized in PPI dendrimer core of low polarity, and the solubility was quantitatively dependent on the molecular weight of the PAMAM dendron and pH. It is a most unique conclusion that the dendrimers effectively act as a favorable doper at high pH along of the different roles of PPI dendrimer core and PAMAM dendron shell, since the hierarchical structure creates a preferable hydrophobic interior. Especially, the phenomenon of pH-dependent solubility is valuable for application as environment-responsible or stimuli-sensitive materials.

Supporting Information Available: Dynamic light scattering results of a PPI(G3)−PAMAM(G3) dendrimer. This material is available free of charge via the Internet at http://pubs.acs.org.

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