Structure-Selective Dye Uptake into an Aggregate of a Copolymer with Linear Polyelectrolyte Block and Hydrophobic Block Carrying Pendant Dendritic Moiety in Water

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Received: September 27, 2004; In Final Form: November 13, 2004

Selective uptake of various dyes into an aggregate of amphiphilic copolymer consisting of a hydrophilic linear polyelectrolyte block and hydrophobic block carrying pendant dendritic moiety has been investigated in water. The copolymer associated into an aggregate with a hydrophobic interior at concentrations above 0.2 mg cm^{-3}. The uptake (23 and 36 molecules per aggregate, respectively) of pyrene and Oil Yellow in an aggregate was one order higher than that of benzo[*a*]pyrene and SudanIII. The hydrophobic dyes are always doped in the interior of the aggregate, but the difference in uptake among dyes may depend on their structure. Even if a large number of guest molecules was doped into the interior of an aggregate, the size of the sphere-like aggregate was conserved. It is suggested that guest molecules are encapsulated into the persisting cavity within and between hydrophobic dendron moieties in an aggregate. Structure-selective uptake reported in this investigation is a unique character of an aggregate of copolymer with dendron moiety because the dendron moiety offers a large void for doping.

Introduction

Amphiphilic molecules including the dendritic moiety have been synthesized, and their physicochemical properties have been examined.[1,2] The first type of such a molecule has a dendritic head and linear chain tail and behaves as a surfactant.[3,4] The second maintains the concentric structure in its interior like typical dendrimers but has an asymmetric hemispherical shell, where both hemispheres have a different affinity to solvent.[5,6] This type of molecule behaves as a sphere-shaped amphiphile. The third type is an amphiphilic polymer with a pendant dendritic moiety. Bo et al.[7,8] have synthesized poly(para-phenylene)s carrying both hydrophobic and hydrophilic dendron side chains at each repeating unit and amphiphilic poly(para-phenylene)s with either pendant dendrons or linear alkyl chains. These are prototypes of an amphiphilic cylinder and have the potential to segregate lengthwise. Their surface-active properties at the air/water interface and on substrate were reported.

We have characterized copolymers consisting of a pendant benzyloxy dendron block and a perfluorinated alkyl side chain block.[9,10] It was confirmed that the copolymers were associated into polydisperse spherical particles in a chloroform solution. The surface pressure−area isotherms of Langmuir monolayers at the air−water interface displayed hysteresis. The molecular orientations at the air−water interface and in Langmuir−Blodgett film were estimated.

In the present work, a copolymer with a linear polyelectrolyte block and a hydrophobic block carrying pendent dendritic moiety was synthesized. It is expected that this polymer forms an aggregate with a hydrophobic interior in water, different from copolymers described previously, and that the aggregate encapsulates small guest molecules in the interior. The dendritic moiety in the interior should play an important part for doping guest molecules because of its available void, which is controlled in structure. Then, the structure-selective uptake of different dyes into the aggregates of the copolymer in water was compared in reference to the structure of the dyes. Moreover, the interior consisting of a polymer block might present a large cavity for doping large amounts of guest molecules. This will provide more superior utilization of polymer aggregates than surfactant micelles as solubilizing agents.

Experimental Procedures

Poly(sodium2-(acrylamido)-2-methylpropanesulfonate)-block-poly(3,5-bis(3′,5′ -bis-(benzyloxy)benzyloxy)benzyl-1,1″-acrylamidoundecanoate) (NaAMPS-*b*-G2) (Figure 1) was synthesized by reversible addition−fragmentation chain transfer (RAFT) controlled radical polymerization[11,12] of 3,5-bis[3′,5′-bis(benzyloxy)benzyloxy]benzyl 11-acrylamidoundecanoate using a sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) based macro-chain transfer agent.[13] 8-Anilino-1-naphthalenesulfonic acid (ANS), pyrene, and 3,4-benzpyrene (benzo[*a*]-pyrene) were purchased from Aldrich Chemical Co. α-(*o*-Tolylazo)-*β*-naphthlamine (Oil Yellow) and 1-(4-(phenylazo)-phenylazo)-2-naphthol (SudanIII) were products of Tokyo Kasei Kogyo and Wako Junsei Kogyo, respectively. Dyes and commercial grade ethanol were used without further purification. Water was purified by distillation and deionization using a Millipore Milli-Q Lab purification system.

Fluorescence spectra were recorded under excitation at 353 nm on a Hitachi F-4010 fluorometer. UV−vis absorption spectroscopic measurements were performed using a Shimazdu UV-2200 instrument. Static and dynamic light scattering (SLS, DLS) were measured at 30 to approximately 150 and 90° scattering angles, respectively, on an Otsuka Electronics DLS-7000DL or DLS-700 spectrophotometer. Cell chambers in the

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10.1021/jp045609r CCC: $30.25
Published on Web 12/31/2004

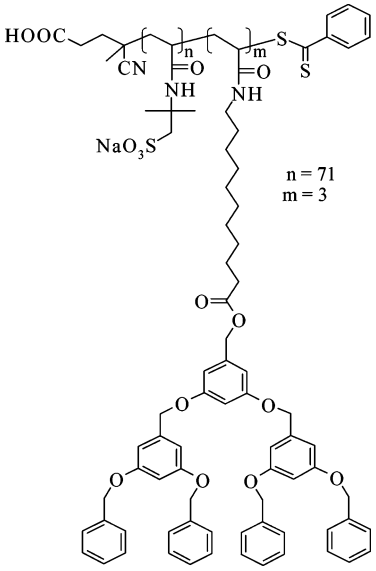

Figure 1. Chemical structure of poly(sodium 2-(acrylamido)-2-methylpropanesulfonate)-block-poly(3,5-bis(3′,5′-bis(benzyloxy) benzyloxy)benzyl-1,1″-acrylamidoundecanoate) (NaAMPS-*b*-G2).

instruments described previously were temperature-regulated at 25 °C during the measurement. Transmission electron microscopic (TEM) photographs were taken on a Hitachi H-7000 microscope. Specimens were prepared by a freeze−fracture replica method at fracture temperature of −120 °C.

Excess solids of dyes were dispersed into an aqueous solution of NaAMPS-*b*-G2 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 NaAMPS-*b*-G2. Separately, aqueous ethanol solutions of NaAMPS-*b*-G2 at 1.0 mg cm^{-3} were also prepared. The molar extinction coefficient (ϵ) of dyes was determined in aqueous ethanol solutions. Then, the molar concentration of dyes in aqueous solutions of NaAMPS-*b*-G2 was calculated from absorbance of a UV−vis absorption band in a dye-solubilized NaAMPS-*b*-G2 solution, which was subtracted from those of dye-nonsolubilized NaAMPS-*b*-G2 solution and dye-solubilized water at corresponding ethanol volume %. Uptake was evaluated as the number of dyes in an aggregate.

Results and Discussion

Determination of Aggregate Formation of NaAMPS-*b*-G2 by ANS Fluorescence. Aggregate formation of NaAMPS-*b*-G2 was determined in an aqueous solution, where a fluorescence probe, ANS, was saturated. NaAMPS-*b*-G2 powders of different amounts were solved in an aqueous solution of ANS, and fluorescence of ANS/NaAMPS-*b*-G2 mixed solutions was measured. As seen in Figure 2A, a fluorescence band of ANS appeared at 520 nm for a NaAMPS-*b*-G2 concentration of 0.005 mg cm^{-3}. An additional band at 420 nm was remarkably observed at 1.0 mg cm^{-3}. Moreover, when the concentration of NaAMPS-*b*-G2 was 2.0 mg cm^{-3}, the strongest band was taken over from 420 to 470 nm.

Figure 2B shows intensities of emission bands at 420, 470, and 520 nm as a function of NaAMPS-*b*-G2 concentration. The intensities of three bands were almost constant at low concentrations but increased at high concentrations except for one concentration (2.0 mg cm^{-3}), where a 420 nm band decreased in intensity. When NaAMPS-*b*-G2 is associated into an aggregate, water-soluble but hydrophobic free ANS is doped into

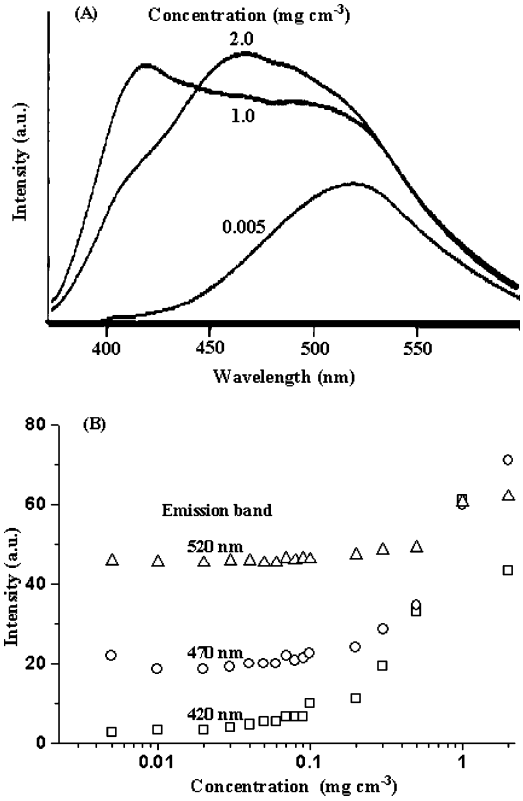

Figure 2. Fluorescence emission spectra of ANS/NaAMPS-*b*-G2 mixtures (excitation: 353 nm). (A) Fluorescence spectra of mixtures at NaAMPS-*b*-G2 concentrations of 0.005, 1.0, and 2.0 mg cm^{-3}. (B) Fluorescence band intensity as a function of NaAMPS-*b*-G2 concentration.

the aggregate because ANS prefers a hydrophobic atmosphere. Free ANS has fluorescence at 520 nm. On the other hand, 420 and 470 nm bands appear and increase their intensities with the progression of aggregation. The slight intensity increase of a 520 nm band at above a 0.2 mg cm^{-3} concentration is only the superposition on tails of the 420 and 470 nm bands. Therefore, it could be determined from Figure 2B that aggregates of NaAMPS-*b*-G2 were formed above 0.2 mg cm^{-3}. There is the change in aggregation at concentrations above 1.0 mg cm^{-3}, which involves the intensity decrease of a 420 nm band. Then, the dye-uptake examination described next was carried out at 1.0 gm cm^{-3}.

Uptake of Dyes in a NaAMPS-*b*-G2 Aggregate. Pyrene, benzo[*a*]pyrene, Oil Yellow, and SudanIII were solubilized in an aqueous solution of NaAMPS-*b*-G2, and the UV−vis absorption spectra of dye-solubilized solutions were compared with the spectra of an aqueous solution of NaAMPS-*b*-G2 and dye-saturated water, as shown in Figure 3. Absorbance of dyes dissolved in water is extremely low, indicating that dyes examined in the present work are hardly soluble. Then, the differential absorbance of a dye-solubilized NaAMPS-*b*-G2 solution to a dye-nonsolubilized NaAMPS-*b*-G2 solution is in proportion to the uptake of dye. The differential absorbance seemed to be larger for pyrene and Oil Yellow than for benzo-[*a*]pyrene and SudanIII.

The uptake of the dye was calculated from the UV−vis absorption spectra in an aqueous ethanol solution, as described in the Experimental Procedures. Table 1 lists parameters used in the calculation (volume % of ethanol, wavelength, and extinction coefficient (ϵ) of a UV−vis absorption band). NaAMPS-*b*-G2 aggregates were characterized from Zimm plot of SLS. Obtained molecular weight, radius of gyration, and virial

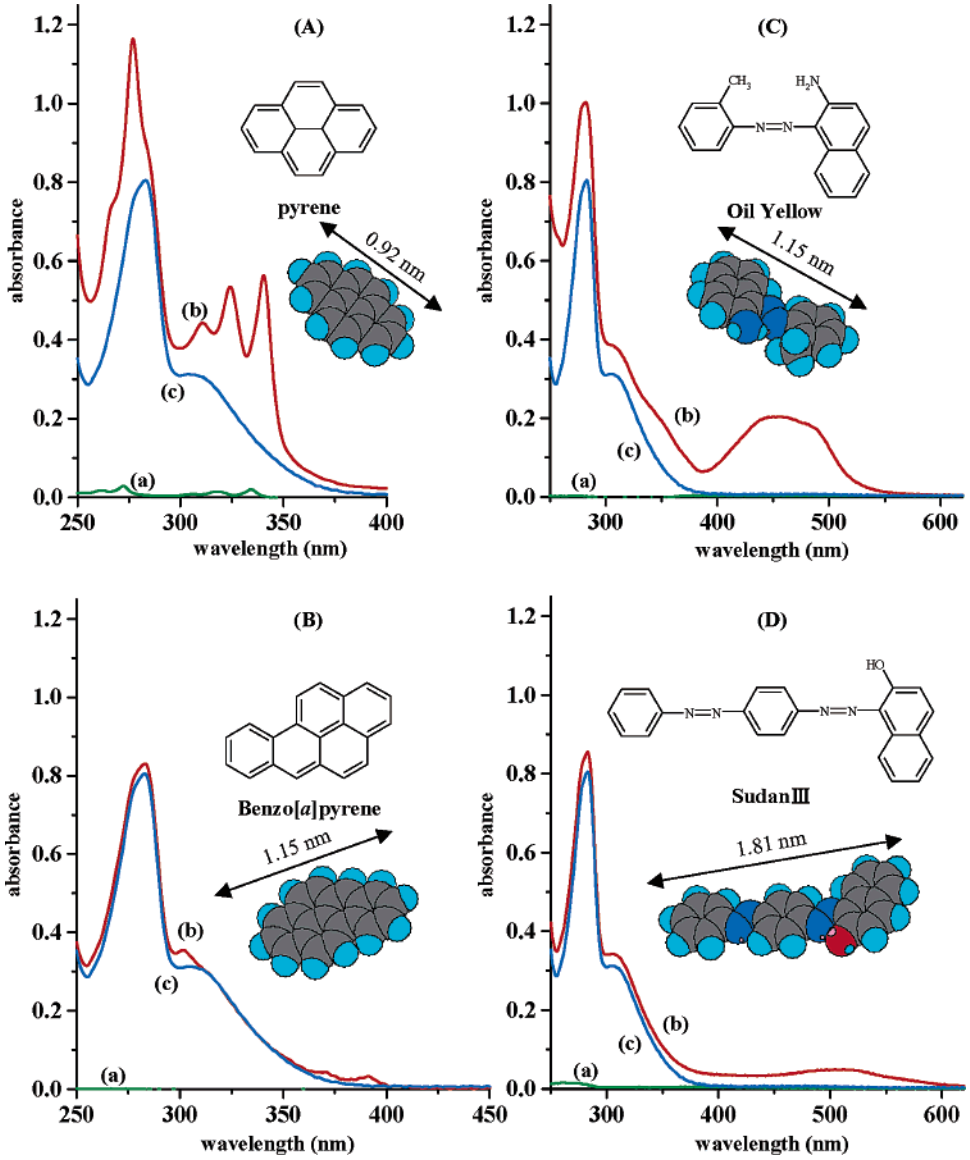

Figure 3. UV−vis absorption spectra of aqueous solutions of NaAMPS-*b*-G2. (A) Pyrene; (B) benzo[*a*]pyrene; (C) Oil Yellow; (D) SudanIII. (a) Dye-solubilized water; (b) dye-solubilized NaAMPS-*b*-G2 solution; (c) dye-nonsolubilized NaAMPS-*b*-G2 solution.

TABLE 1: UV−vis Absorption Band of Dye and Number of Dye Doped in NaAMPS-*b*-G2 Aggregate

dye	concentration of EtOH/volume %	UV−vis absorption band		no. of doped dye per NaAMPS-*b*-G2 aggregate
		wavelength/nm	$\epsilon/10^4$ M^{-1} cm^{-1}	
pyrene	40	334.8	4.40	22.9
benzo[*a*]pyrene	50	384.5	3.38	2.7
Oil Yellow	50	442.5	1.69	35.9
SudanIII	80	509.5	4.33	3.4

coefficient were 2.60×10^6, 26.2 nm, and 1.04×10^{-5} cm^3 mol g^{-2}, respectively. Then, the aggregation number of NaAMPS-*b*-G2 is 104. By utilizing this aggregation number, the number of doped dye per NaAMPS-*b*-G2 aggregate is evaluated and listed in Table 1.

The uptake of pyrene and Oil Yellow was one order higher than that of benzo[*a*]pyrene and SudanIII. All dyes take a planar shape, and major axes of pyrene, benzo[*a*]pyrene, Oil Yellow, and SudanIII are about 0.9, 1.2, 1.2, and 1.8 nm, respectively, as illustrated in Figure 3. Since pyrene is a small molecule, many pyrene molecules can be doped in the NaAMPS-*b*-G2 aggregate. On the contrary, SudanIII is so big that it is encapsulated. Meanwhile, despite almost the same major axis of Oil Yellow and benzo[*a*]pyrene, Oil Yellow was solubilized more easily than benzo[*a*]pyrene. Moreover, a larger amount of Oil Yellow is doped rather than pyrene. Oil Yellow consists of two small volume parts connected by an azo group, while benzo[*a*]pyrene and pyrene take a discoid shape. The former structure should be more fit in the complicated void in the polymer aggregate. Thus, the difference in the configurational structure of the dye may raise the difference in doping.

Characterization of Pyrene-Doped NaAMPS-*b*-G2 Aggregate. The micropolarity around the solubilization site in the dendrimer can be evaluated as an emission intensity ratio, I_3/I_1, of the third to the first monomeric band in a pyrene fluorescence spectrum, which is known as a polarity index.[14] While the I_3/I_1 of pyrene is ~0.64 in water, namely, in the hydrophilic environment, it increases with increasing hydrophobicity, and the value is 0.70~1.00 in surfactant micelles and 1.65 in pure hydrocarbon solvents. The I_3/I_1 value evaluated from a fluo-

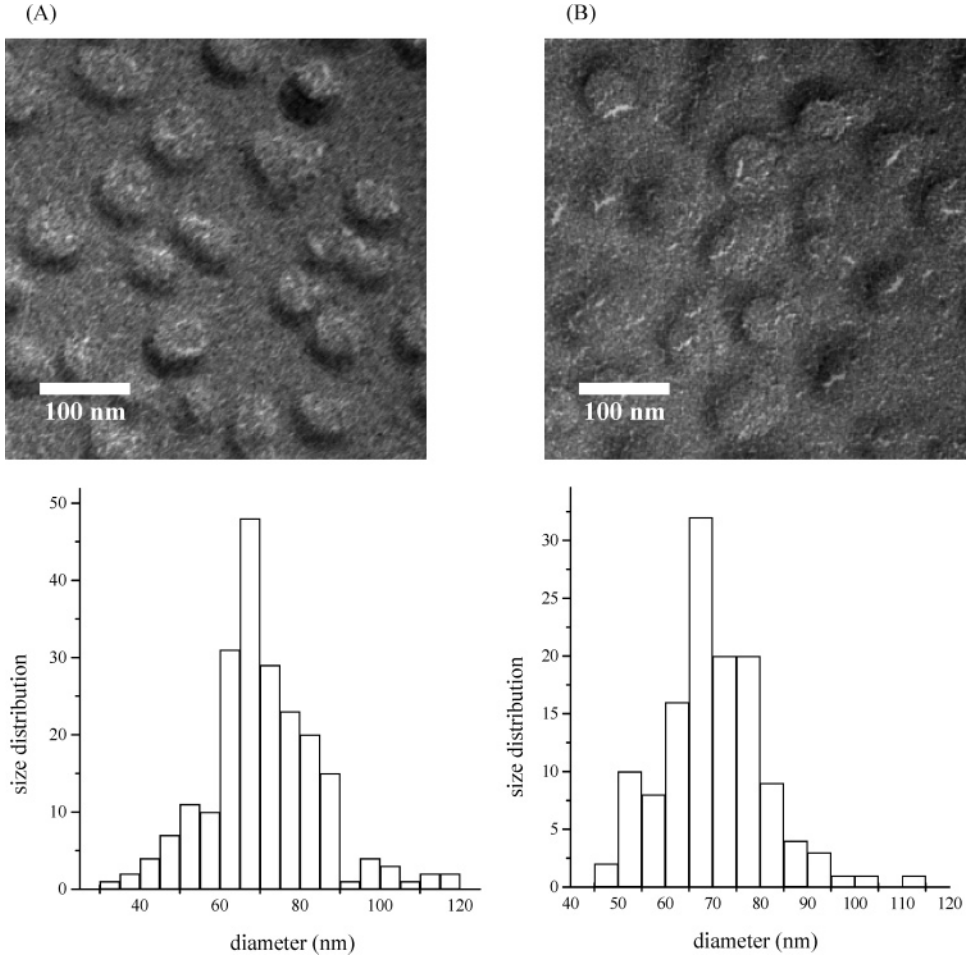

Figure 4. TEM photograph (top) and size distribution (bottom) of NaAMPS-*b*-G2 aggregates in water (1.0 mg cm^{-3}). (A) Pyrene-undoped and (B) pyrene-doped.

rescence spectrum of pyrene in a NaAMPS-*b*-G2 aggregate was 0.75. It is suggested that pyrene exists in a hydrophobic environment of the NaAMPS-*b*-G2 aggregate. Such an environment should be formed by the benzyloxy dendron side chain in the interior of the aggregate. Thus, it was confirmed that the NaAMPS-*b*-G2 aggregate dopes pyrene interiorly. The polarity index in the NaAMPS-*b*-G2 aggregate should be compared with those (0.5∼0.6) of pyrene in poly(amide amine) and poly(propyleneimine) dendrimers in water, which indicates the location of pyrene in the hydrophilic environment in dendrimers.[2]

Size and morphology of the NaAMPS-*b*-G2 aggregate were obtained from DLS and TEM, and they were compared with those of the pyrene-solubilized aggregate. Although no aggregates were found at concentrations below 0.2 mg cm^{-3}, images of sphere-like particles were observed above this concentration, as shown in Figure 4, which includes histograms of size distribution. The averaged particle size of 70.5 ± 0.3 nm in diameter changed scarcely, even if pyrene was doped. The hydrodynamic diameter (64.4 nm) evaluated from DLS was also not different between before and after doping of pyrene. This result was no inconsistency with TEM examination, although it should be noted that the diameter from TEM is slightly larger than that from DLS. One of the reasons is the inaccuracy of the size determination through photography, and another is the flattening of the soft aggregates during the preparation of the dried specimen for TEM. No change of aggregate size after the dye was doped indicates that dyes were doped in the persisting void in an aggregate but not in the newly created space. Such a persistant void should be created by the cavity within and between dendron moieties since dyes are doped in the hydrophobic interior formed by dendron moieties.

Conclusions

An amphiphilic copolymer consisting of a linear polyelectrolyte (hydrophilic) block and a pendant dendron (hydrophobic) block associated into an aggregate with hydrophobic interior in water. The aggregate doped hydrophobic dyes in the interior. Then, the uptake of dyes into the aggregate of the copolymer in water was affected by the chemical structure and size of dyes. The size of the sphere-like aggregate was conserved without change, even if a large number of guest molecules (23 for pyrene and 36 for Oil Yellow) was doped into the hydrophobic interior of an aggregate. This indicates that guest molecules are encapsulated into the persisting void in an aggregate, which was created by the cavity within and between dendron moieties. The doping behavior reported in the present paper can be compared with that of surfactant micelles, where very few molecules are solubilized into spherical micelles.[15,16]

Acknowledgment. We are grateful to Shiseido Materials Science Consortium for its financial support.

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