Fluorescence Investigations of Oxygen-Doped Simple Amine Compared with Fluorescent PAMAM Dendrimer[a]

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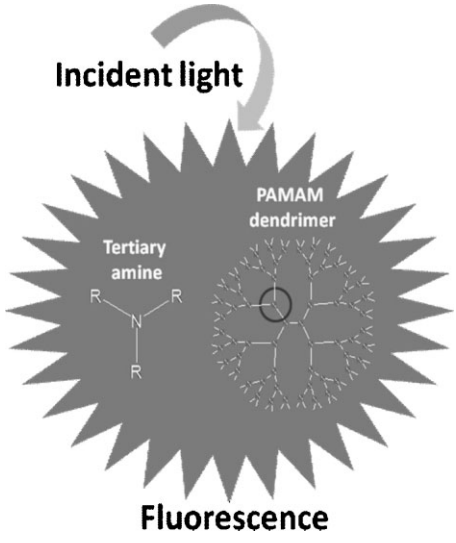
Blue emission of oxygen-doped tertiary amine (triethylamine), a key unit of fluorescent poly(amido amine) dendrimer, was demonstrated. It was found that the fluorescence intensity could be further enhanced if the tertiary amines locate densely in the dendrimer interior as the branching sites. Moreover, a solvatochromic phenol blue, instead of oxygen, is able to induce the blue fluorescence of the tertiary amino-branching sites based on a guaranteed host-guest complexation of phenol blue molecules and dendrimer interior.



Introduction

A blue photoluminescence of dendrimers, produced from 1 → 2 *N*-branched dendrimers and hyperbranched polymers, has been reported.[1–8] Bard's group[1] claimed that the oxidation of hydroxyl end-groups on poly(amido amine) (PAMAM) dendrimers was the main contribution to the special fluorescence phenomenon. However, in addition to PAMAM dendrimers with different end-groups,[2,5] the authors' group[5,7] reported that commercially-available poly(propyleneimine) (PPI) dendrimer and synthesized poly(ethyleneimine) (PEI) dendrimer, containing only alkyl amine units in the dendritic structure, can also emit a blue fluorescence after similar air treatment. Although some reports indicated a pH dependency, such that acidic conditions lead to a higher emission intensity than basic conditions,[2,5,6] the photoluminescence of polysiloxane coated by PAMAM dendron side chains was intensified in crowded circumstances under alkaline condition, rather than in less-crowded circumstances under acidic conditions.[4] These results imply that the more rigid, crowded dendritic structure – attributed to the interior tertiary amines – exhibits a higher fluorescence yield. Therefore, these observations motivate us to examine the fluorescence of simple amines, because it is speculated that the tertiary amino moieties at the branching sites inside the dendritic backbone play a key role for this special fluorescence phenomenon of all the 1 → 2 *N*-branched dendrimers and hyperbranched polymers.

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[a] Supporting information for this article is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at http://www.mrc-journal.de, or from the author.

Experimental Part

Sample Preparation

Two methods were introduced for the preparation of fluorescent simple amines and dendrimers in water. Air-purged samples were

DOI: 10.1002/marc.200800571

prepared by continuously stirring at room temperature under an air atmosphere for 7 days. Alternatively, mixtures that contains 0.2 cm^3 of either triethylamine (TEA) or fourth generation (G4) PAMAM dendrimer with NH_2 terminals (10 wt.-% in methanol), 0.2 cm^3 of aqueous ammonium persulfate (APS, 0.1 M), and 2.6 cm^3 of water were stirred for 3 days at room temperature to yield APS-treated samples. A stock solution of phenol blue (PB) (1×10^{-3} M) was prepared by dissolving the dye in tetrahydrofuran (THF) and stored in the dark. The preparation of PB-doped dendrimer samples was carried out by dropping 2 mm^3 of the PB stock solution into 1 cm^3 of an aqueous solution (0.1×10^{-3} M) of either untreated or fluorescent dendrimer. Ultra-high purity nitrogen gas was then introduced to strip off trace amounts of THF, and the mixed solutions were gently stirred at room temperature for 72 h to facilitate dye solubilization in the dendrimer.

Instruments

UV-vis absorption and steady-state fluorescence spectra were recorded in a quartz cuvette (1 cm path length) on a Shimadzu BioSpec-1600 UV-vis spectrometer and a Hitachi F-3010 fluorescence spectrophotometer, respectively. Steady-state anisotropy data were collected at 25 °C in "L"-format using two UV-vis polarization filters ($0 \rightarrow 90°$) for excitation and emission exits. ^1H (300 MHz) and ^{13}C-NMR (75 MHz) spectra were performed by Jeol JNM-CMX300, and Fourier transform IR (FT-IR) spectra were recorded on Varian FT-60A/896. Elemental analysis was carried out by a PerkinElmer 2400 series II CHNS/O elemental analyzer. *N*-phenylacetamide was used as the calibration standard, and the measurement for each sample was repeated three times. Optical and fluorescence microscopic images of dendron surface-modified silica particles were taken by a Nikon Eclipse TE2000-U, equipped with a 20 ×/0.50 objective lens, a super high pressure mercury light source and a DS camera control unit DS-U1.

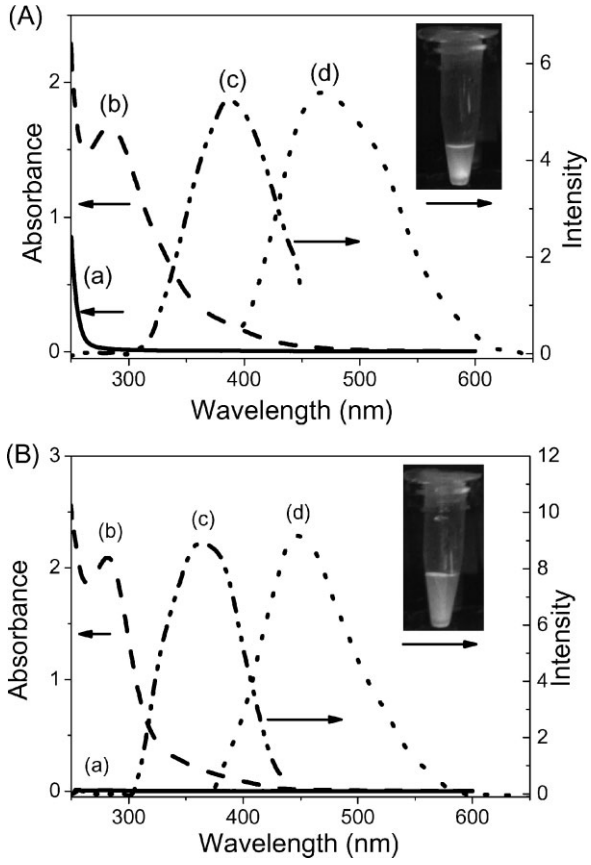

Figure 1. A) UV-vis spectra of fresh (a) and APS-treated (b) TEA (2 M); excitation (c) and emission (d) spectra of APS-treated TEA; $\lambda_{ex} = 365$ nm, $\lambda_{em} = 460$ nm. B) UV-vis spectra of fresh (a) and APS-treated; (b) G4 PAMAM dendrimer (1×10^{-3} M); excitation (c) and emission (d) spectra of APS-treated G4 PAMAM dendrimer; $\lambda_{ex} = 365$ nm, $\lambda_{em} = 450$ nm.

Results and Discussion

TEA was selected as a typical tertiary amino-branching point inside PAMAM, PPI (or PEI) and poly(amino ether) dendrimers/polymers. Colorless aqueous solutions of TEA and G4 PAMAM dendrimer gradually turned pale yellow and emitted a blue fluorescence after treatment by APS for 3 d. Figure 1A and B shows the excitation and emission spectra of APS-treated TEA and dendrimer, respectively. Both as-prepared TEA and dendrimer solutions emit blue fluorescence in the spectral range of 440 to 460 nm under excitation at 365 nm. This result strongly supports the assumption that the tertiary amino-moieties inside the dendrimer are involved in the fluorescence phenomenon. In addition, UV-vis absorption spectra of APS-treated TEA and dendrimer (Figure 1), compared to those of fresh TEA and dendrimer, show an obvious absorption band centered at 280 nm; It should be noticed that no fluorescence emission is observed under 280 nm excitation but the emission occurs at the 365 or 400 nm excitation. The π-π^* transition of substituted aromatic compounds usually contributes to the absorption around 280 nm, but such aromatic structures do not exist in TEA and G4 PAMAM dendrimer molecules.

APS-treated TEA exhibits a much lower fluorescence quantum yield (0.25%) than APS-treated dendrimer (52%), when it is determined using quinine sulfate as a reference. Since one dendrimer molecule consists of 62 tertiary amino-branching points, the fluorescence intensity per tertiary amino unit of as-prepared dendrimer is 3.4 times stronger than that of TEA. The result implies that the as-prepared tertiary amines exhibit higher fluorescence quantum yield inside the dendrimer than in free dispersion in the aqueous solution. In other words, the fluorescence intensity could be enhanced, if the tertiary amines located densely in the dendrimer interior. There are two possible reasons. Firstly, air- or APS-treatment of the tertiary amines inside dendrimer could be more efficient than that in bulk of an aqueous solution, because the dendrimer can effectively encapsulate oxygen or oxidant molecules by host-guest affinity. Secondly, it is

Macromol. Rapid Commun. **2009**, *30*, 89–93

Macromolecular Journals

DOI: 10.1002/marc.200800571

supposed that the active species, concentrated and restricted in a confined dendrimer "pocket", causes the fluorescence enhancement effect due to lack of rotational and translational freedom. A simple anisotropy measurement showed a higher value of fluorescent PAMAM dendrimer (0.13) than of fluorescent TEA (0.02), indicating free TEA molecules exhibit a lower fluorescence polarization effect. Since fluorescence anisotropy is directly dependent on rotational correlation time, the result clearly supports the rotational motion of tertiary amino moieties generating the blue emission being restricted inside the dendrimer.

^{1}H-NMR of fresh TEA molecule (Figure S1, in Supporting Information) shows the triplet CH_3 and quartet CH_2 signals (A_3X_2 splitting pattern) at $\delta = 0.97$ and 2.46 ppm, respectively. Moreover, ^{13}C-NMR shows the CH_3 and CH_2 signals at $\delta = 11.5$ and 46.2 ppm, respectively. However, the NMR spectra of fluorescent TEA (Figure S1) exhibit the same characteristic proton and carbon signals as those of fresh TEA. This result excludes the formation of new chemical structure after the APS treatment of TEA. In addition, the fact that the FT-IR spectra of TEA before and after treatment (Figure S2, in Supporting Information) show almost identical characteristic vibration bands also supports the mention from NMR examination.

It is known that saturated amines are electron donors due to lone-pair electrons on the nitrogen atom and, therefore, in some PAMAM or PPI (and PEI) dendrimer/dye complexes, fluorescence of the incorporated dye molecules could be quenched by the tertiary amino-branching units through a reductive electron-transfer process.[9] In addition, one important feature of tertiary amine, among all the saturated amines, is the possession of a significant fluorescence quantum yield and considerable stokes shifts in the vapor phase.[10] However, this study demonstrates that the TEA solution, upon APS treatment, emits blue fluorescence away from the intrinsic emission wavelength of 280–320 nm for pure tertiary amine.

Since the oxidation process does not really occur under ordinary reaction conditions, the generation of O_2 by the oxygen-abundant molecule (APS) should be considered. In this case, it can be assumed that the fluorescence is attributed to the formation of an "exciplex" between a tertiary amine and a doped O_2 molecule. Once the lone-pair electrons of tertiary amine or oxygen are excited through either incident light or electrochemical activation, two species may form the exciplex, driving the intrinsic emission of tertiary amine to longer wavelength. Jayamurugan et al.[11] found inherent photoluminescence of a newly-established poly(propyl ether imine) dendrimer, bearing tertiary amines as branching sites and ether as linkers. They also emphasized that the oxygen in-built within the dendritic structure easily interacts with tertiary amines, leading to the emission behavior without any oxygen from an external source.

Another possibility is the formation of a peroxyl radical, originated from tertiary amine and oxygen. Lalevée et al.[12] reported a direct observation of particular peroxyl radical (TEA–$O_2^\bullet$) by laser flash photolysis and demonstrated a strongly red-shifted transient absorption band of TEA–$O_2^\bullet$ at ≈380 nm in comparison with that of aminoalkyl radical (TEA$^\bullet$) at ≈320 nm. The transient absorption band of this adduct radical species quite closes to the excitation wavelength of APS-treated TEA and 1 → 2 *N*-branched dendrimer. Therefore, it is also considered that a short-lived radical species such as tertiary amine-$O_2^\bullet$ could undergo the absorption of near visible light, and then fast relaxation gives the blue fluorescence.

Since an O_2 molecule, generated by either air or APS, could be the dopant (guest) for the simple amines and dendrimers (host), it may be substitutable by other oxygen-containing molecular probes, in which spectroscopic measurements for monitoring the special host-guest complex are easily carried out. Herein, phenol blue (PB) with a solvatochromic property, was selected as the probe molecule based on the following characteristics: 1) the size of PB is smaller than common organic dyes, which guarantees the match to the interior spaces of dendrimers; 2) PB's solvatochromic absorption band is in the visible region from 550 to 660 nm and blue-shifts, as the environmental polarity decreases; and, 3) PB also has a solvatochromic fluorescence band in the less-polar environment, being far from the emission of fluorescent tertiary-amine branched dendrimer. It has been reported that a PB probe can be well doped within the interior of PAMAM dendrimers but not on their surface (or periphery) and that the blue-shifted absorption band of PB encapsulated in PAMAM dendrimer indicates a less polar microenvironment inside the dendrimer.[13]

Once fresh G4 PAMAM dendrimer was doped by PB guests, the mixing solution shows a visual color change from blue to purple, indicating a successful encapsulation. The UV-vis spectra (Figure 2) clearly reveal the blue-shifted absorption band of PB after 3 d. Meanwhile, PB emitted a red fluorescence at 570 nm upon excitation at 550 nm, whereas no fluorescence could be observed for an aqueous PB solution (without dendrimer). It is mentioned that PB hardly emits any fluorescence in solvents with high polarity and dielectric constant, such as water or trifluoroethanol, but emits an apparent red fluorescence in the less polar solvent, such as furan ($\lambda_{max} = 562$ nm).[14] Therefore, the present spectroscopic data is indicative of a successful association between PB and the dendrimer interior in water, resulting in the appearance of red emissions from the PB probe due to its solvatochromic characteristics in the relatively non-polar microenvironment of the dendrimer interior.

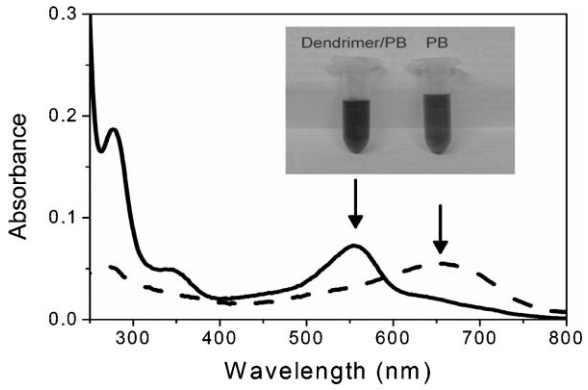

Figure 2. UV-vis spectra of aqueous PB solution (2×10^{-6} M) and dendrimer/PB complex solution (dendrimer: 0.1×10^{-3} M). The absorption maximum shifted from 650 to 550 nm after complexation.

The probe molecule was assured, as described above, to be close-packed in the dendrimer cavity, based on a blue-shifted absorption band and an appearance of the red emission band (590 nm) of PB. Therefore, electron-rich PB molecule with quinone-like and *N,N*-dimethylamino heads may induce the emission of dendrimer. Indeed, fluorescence of PAMAM dendrimer upon excitation at 365 nm gradually shifted from weak intrinsic band at 440 nm to a stronger band at 490 nm. As shown in Figure 3A and D, the fluorescence intensity increased for both dendrimer host and PB guest up to 7 d, although the emission pattern of the dendrimer without PB treatment only slightly changed after 7 d. It is clear that PB, rather than O_2, actually induces the fluorescence of dendrimer, and that complexation in the interior of dendrimer guarantees a short distance between PB and the tertiary amino-branching unit, leading to the effective collision and formation of exciplexes or radicals with a red-shifted emission of tertiary amino units.

In the case of air and APS-treated G4 PAMAM dendrimer, fluorescence was already induced by the encapsulation of O_2 molecules in the interior of the dendrimer, although fluorescence of APS-treated dendrimer is more intense than that of air-treated dendrimer (Figure S3, in Supporting Information). When a PB probe is added to two fluorescent dendrimer solutions, the visual color of the solutions changed to violet. This suggests the successful complexation of PB and dendrimer. Meanwhile, as seen in Figure 3B, the emission band of air-treated dendrimer also gradually shifted to a longer wavelength (440 to 470 nm); however, this shift is not comparable to the case of untreated dendrimer. Since the interior of air-purged dendrimer is not fully occupied by O_2 molecules, the enhancement of dendrimer emission is allowed by

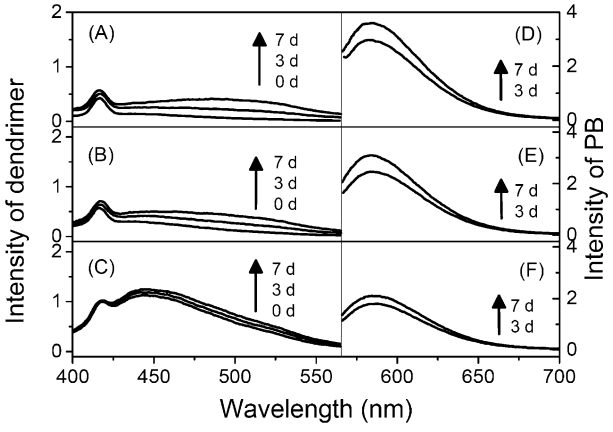

Figure 3. Emission tracing of G4 PAMAM dendrimer (0.1×10^{-3} M) after mixing with PB (2×10^{-6} M) for 0, 3 and 7 d. Dendrimer: A,D) fresh, B,E) air-treated, C,F) APS-treated. Excitation: A–C) $\lambda_{ex} =$ 365 nm, D–F) $\lambda_{ex} = 550$ nm. The peak centered at 415 nm is attributed to the Raman scattering of H_2O molecules upon excitation at 365 nm.

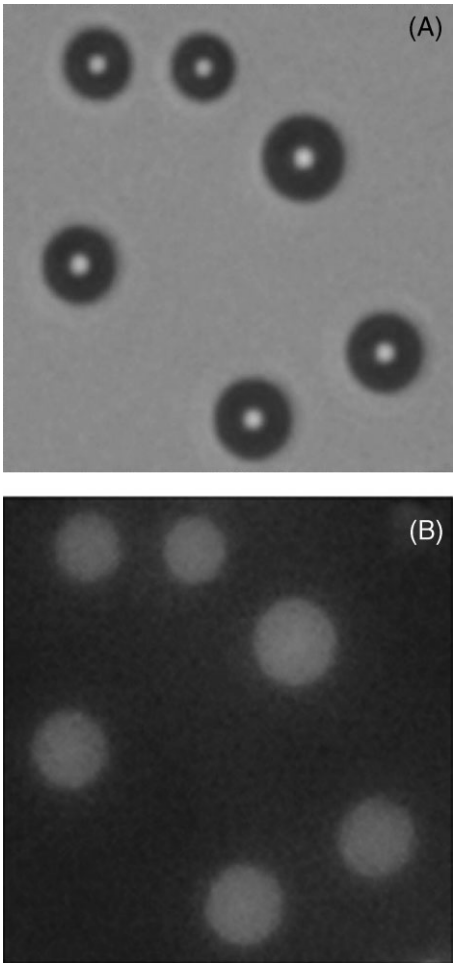

Figure 4. A) Optical and B) fluorescence microscopic images of G3 PAMAM dendron surface-modified silica particles; $\lambda_{ex} = 365$ nm.

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DOI: 10.1002/marc.200800571

additionally encapsulated electron-rich PB. Moreover, it is noticed that the emission band of APS-treated dendrimer remains nearly invariable after encapsulation of PB probe (Figure 3C). This supports, once the dendrimer was doped by O_2 molecules until saturation, the formation of the second complexation composed of PB and tertiary amino-branching site being impossible. The spectroscopic data not only suggests that both O_2 and PB can be the guest molecules for the hosts of interior tertiary amine, but also implies that the successive complexation of dendrimer with these two dopants is a competitive process. It is noted that the emission intensity of PB at 590 nm (Figure 3D–F) reflects the doped amount of PB as a sequence of fresh > air-treated > APS-treated dendrimer, corresponding to the sequence of interaction between PB and tertiary amino-branching site.

Finally, even if PAMAM dendrons are immobilized on solid phase through stepwise synthetic procedures, as in the case of G3 PAMAM dendron surface-modified porous silica particle,[15] these particles could emit a strong blue fluorescence, making fluorescent silica particles as shown by microscopic images (Figure 4). Then, it is suggested that the laboratory-made PAMAM dendrons without any post-treatment (APS, air, and PB) directly contribute to the blue fluorescence of the silica particles.

Conclusion

It was shown that TEA, upon doping APS (O_2 molecule generator), radiates similar blue emission to fluorescent PAMAM dendrimer. Moreover, for PAMAM dendrimer, the doping of solvatochromic PB molecules (oxygen-containing molecular probe), which are guaranteed to be encapsulated in the interior of the dendrimer, leads to similar blue emissions. Besides the solution methods, fluorescent porous silica particle, on which PAMAM dendrons are surface-grafted, has also been developed.

Our effort indicates that the fluorescence property of $1 \rightarrow 2$ *N*-branched polymers originates from the interior tertiary amino-branching sites, and that O_2 or oxygen-containing molecules are essential for this. It can be assumed that both TEA and *N*-branched polymers can form complexes (either peroxyl radical or exciplex) between oxygen and tertiary amino-branching site, leading to transient absorption and blue emission of tertiary amine.

Supporting Information available: NMR analyses for TEA and additional emission spectrum.

Received: September 11, 2008; Revised: October 28, 2008; Accepted: October 29, 2008; DOI: 10.1002/marc.200800571

Keywords: blue emission; dendrimer; fluorescence; oxygen; phenol blue; poly(amido amine) dendrimer; tertiary amine

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