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Enhancement of optical properties of dyes for bioprobes by freezing effect of molecular motion using POSS-core dendrimers

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ABSTRACT

We demonstrate that the POSS-core dendrimer induced various kinds of favourable properties of trisvinyl-pyridinium triphenylamine (TP3PY) as a bioprobe. By using the amphiphilicity of the POSS core, the complexes of TP3PY with G2 POSS-core dendrimer were prepared, and the series of properties were investigated for the application as a bioprobe. Initially, it was shown that the adsorption of TP3PY onto the vessels was highly prohibited by the complex formation with the dendrimers. The solution states of the dendrimer complexes were maintained at least for 7 days. Moreover, it was found that the improvement of quantum yields and the elongation of fluorescent lifetimes were observed by the complexation with the dendrimers. Similar photochemical properties were obtained in a glassy state of 2-methyltetrahydrofuranat −196 °C. The molecular rotations occurring at the excited state could be restricted by the complex formation with dendrimers. These characteristics induced by the complexation with the POSS-core dendrimer are of significance to improve the signal to noise ratio and the accuracy on the detection as a bioprobe.

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1. Introduction

The optical materials which show the strong emission in deep red or near-infrared regions are strongly required in bioimaging.[1] Since the vital body shows higher tolerance and lower self-emission, the background noise and the autofluorescence of the samples can be suppressed and the optical detection is applicable for the detection at deep spots inside vital bodies.[2] The series of electron-withdrawing group-conjugated triphenylamine derivatives are known as the fluorophore with the strong emission in the deep red region.[3] Moreover, the branched donor–acceptor skeleton shows non-linear optical properties, resulting in the large applicability for multi-photon excitation.[3] However, the poor water-solubility and the strong adsorption ability limit to the conventional usages as a bioprobe.

Dyes located at the interfaces or in the solid matrices often show the attractive optical properties for bioprobes. For example, the lipophilic molecules can be readily adsorbed onto the silica materials composed of the low dielectiric Si–O–Si bonds via strong hydrophobic interaction. Thus, in the dye-loaded glass materials, the rigid silica frameworks can inhibit the aggregation by isolating each molecule and maintain the well-dispersion state of the dyes in the matrices, resulting in the suppression of the self-quenching.[4] Consequently, the enhancement of the emission quantum yields or the elongation of fluorescence life times was observed.[4] These phenomena are applicable to improve the signal to noise ratio in the images in the optical methods using the dye as a marker for bioimaging. Okamoto et al. presented that the molecular rotations of the dimethylaminopyrene-tethered DNA base at the excited sate were regulated by the duplex formation of DNA.[5] Multi-colour emission was observed from the aqueous solutions containing the modified DNA oligomer at the ambient temperature. These phenomena improved the accuracy on the detection by correlating the one emission signal from another one at a different wavelength. Such characteristics are promised to be a key for developing advanced optical probes. However, there are problems to apply these characteristics to develop a bioimaging probe. It is difficult to realize these properties with the dye-loaded materials under biological conditions and particularly maintain the well-dispersion states of the dye/matrix complexes without loss of the induced properties. Furthermore, the size-distribution of the complexes inhibits the precise controls of the locations of the complexes in vivo. In order to produce the sophisticated probes, these problems should be solved.

Polyoctahedral oligomeric silsesquioxanes (POSS, Fig. 1) have been used as building blocks not only for functional nanomaterials but also for biomaterials.[6] The typical POSS molecule possesses a cubic rigid structure represented by the formula $R_8Si_8O_{12}$, where the central inorganic core (Si_8O_{12}) is functionalized with organic moieties (R) at the eight vertices. POSS can be regarded as a kind of glass materials. Therefore, the luminescence of the POSS core was hardly observed.[7] On the other hand, the modified POSS with the chromophores showed attractive characteristics.[8] Moreover, POSS had a low dielectric constant due to the closed silica structure.[9] Thereby, the water-soluble POSS-core dendrimers showed the amphiphilicity and the entrapping ability of various

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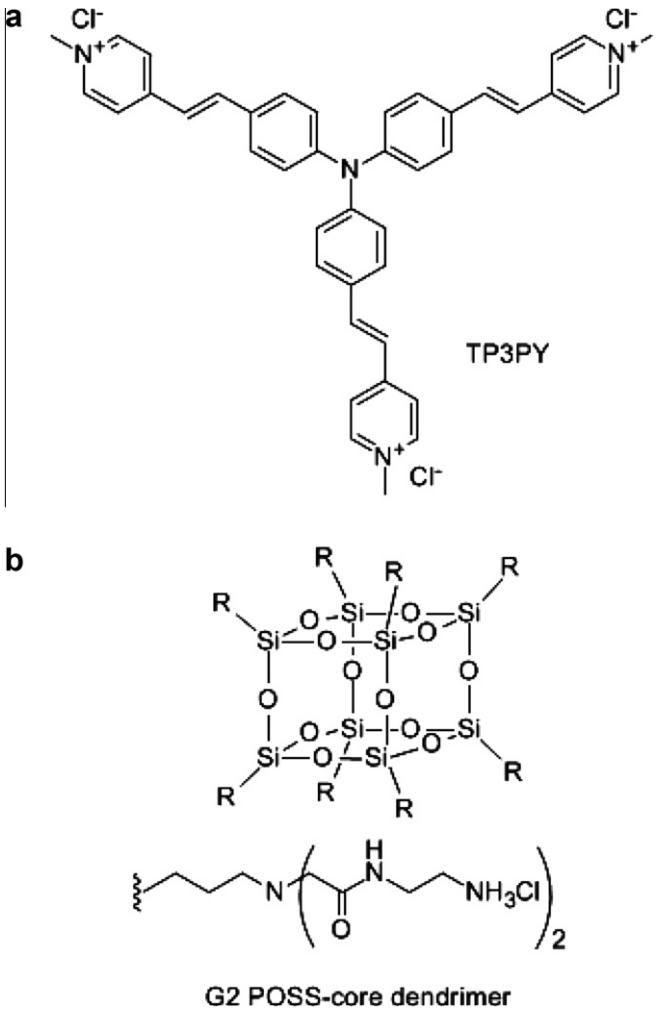

Figure 1. Chemical structures of the dye and the dendrimer used in this study.

kinds of guest molecules.[10,11] Compared to the same generation of the polyamidoamine dendrimer, it was found that a larger amount of guest molecules were encapsulated with high affinity.[10] Next our interests have directed to regulate the molecular rotation of the dyes by encapsulating into the POSS-core dendrimers similarly used as the bulk silica materials.[11]

Herein, we report that the POSS-core dendrimer can induce various kinds of favourable properties of trisvinyl-pyridinium triphenylamine (TP3PY, Fig. 1)[3a] as a bioprobe. We encapsulated TP3PY into G2 POSS-core dendrimer and evaluate the properties for applying the dendrimer complexes. The undesired adsorption of TP3PY onto the vessels was highly prohibited by encapsulation. In addition, the improvement of quantum yields and the elongation of fluorescent lifetimes were observed by encapsulating into the dendrimers. Similar photochemical properties were realized in the frozen glassy matrix of 2-methyltetrahydrofuranat – 196 °C. This is, to the best of our knowledge, to show the dendrimer can show the freezing effect on the molecular rotation of the encapsulated molecule and accomplish to express the favourable optical properties under biocompatible circumstances.

2. Experimental section

2.1. General

\The UV–vis absorption spectra were obtained on a SHIMADZU UV-3600 spectrometer. Emission spectra of the samples were monitored by a Perkin–Elmer LS50B fluorometer using a 1 cm path length cell. The excitation bandwidth was 0.1 nm. The emission bandwidth was 0.1 nm. Fluorescence lifetime analysis was carried out on a HORIBA FluoreCube spectrofluorometer system; excitation at 375 nm was carried out using a UV diode laser (NanoLED-375L).

2.2. Synthesis

TP3PY was synthesized according to the previous report.[3a]Synthetic protocols of G2 POSS-core dendrimer are shown in the Supplementary data.

2.3. Complexation with the dendrimers

General procedure for the complexation of TP3PY by the dendrimers is described here. The stock solutions of TP3PY (×10) in methanol and G2 POSS-core dendrimer (×10) in water were mixed at room temperature, and then the 500 μL of the samples were prepared by adding the solvents.

2.4. Evaluation of the amount of the retained ligand into the G2 POSS-core dendrimer

The sample solutions were stored under ambient conditions and filtered through Nanosep 3 K centrifugal devices (Pall Life Sciences) by centrifugation (2000*g*, 30 min, 25 °C). The concentration of TP3PY was determined from the light absorption of the filtrates. The amounts of retention were evaluated by comparing to the absorptions of the filtrates.

2.5. Fluorescence measurements of the complexes

The fluorescence emission of TP3PY (10 μM) under excitation at 474 nm was monitored at 25 °C using 1 cm path length cell. The excitation bandwidth was 1 nm. The emission bandwidth was 1 nm. The quantum yields were determined as an absolute value with an integral sphere.

3. Results and discussion

TP3PY and G2 POSS-core dendrimer were synthesized according to the previous reports.[3a,12] The solution samples were prepared by adding the stocked solution of TP3PY in methanol into that of G2 POSS-core dendrimer in water and adjusting the desired concentration with the solvents.To monitor the dispersibility of the complexes in PBS, the samples in the quartz vessels were stored at 25 °C in the dark, and then further treatments were carried out. The fluorescence spectra were obtained with the excitation light at 474 nm. The quantum yields of fluorescence emission from TP3PY (Φ) were determined as an absolute value with an integrating sphere.

To confirm the encapsulation of TP3PY into the dendrimer and to evaluate the essential stoichiometry of G2 POSS-core dendrimer for solubilizing TP3PY in PBS, we compared the absorption of the supernatants obtained from the solutions containing TP3PY with various concentrations of G2 POSS-core dendrimer after the storage under ambient conditions (Fig. 2).[13] If the solution state is maintained, the concentration of the dissolved TP3PY should be less affected. Based on this idea, we investigated the absorption changes of the supernatants after 3 days. The solutions containing 10 μM TP3PY in PBS with various concentrations of G2 POSS-core dendrimer were prepared, and the centrifugation was treated with the solutions after 3 days. The supernatant of the pure TP3PY provided significant lower absorption with the peak at 474 nm after 3 days. In contrast, the degree of the decrease of the absorption from the supernatant reduced corresponding to the increase of

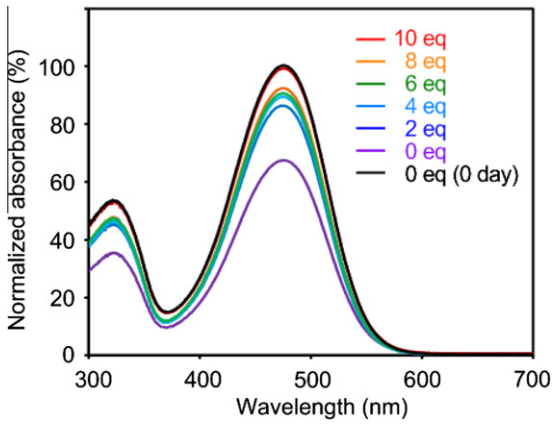

Figure 2. The absorption changes of TP3PY in the filtrates with various ratios of G2 POSS-core dendrimer to TP3PY. The samples in PBS were stored at 25 °C for 3 days in darkness and passed through the size-exclusive filter.

the concentration of G2 POSS-core dendrimer. It was found that the decreases of the absorption were completely suppressed in the presence of 10 equiv. From the dynamic light scattering experiments, the significant values were less obtained over the detectable region (2 nm∼). These facts suggest that 10 equivalents of G2-POSS core dendrimer are necessary to maintain the solution state of TP3PY. The branched and larger size of TP3PY than that of the POSS-core dendrimer might require an excess amount of the dendrimer for the stabilization of the solution state. We added the 10-fold concentration of G2 POSS-core dendrimer for following experiments to maintain the well-dispersion. The emission intensities of TP3PY were enhanced even after 3 days incubation by the encapsulation. The photochemistry of TP3PY in the dendrimers will be mentioned as below.

The complex stability of G2 POSS-core dendrimer with TP3PY was examined. The solutions containing 10 μM TP3PY in PBS with or without 100 μM G2 POSS-core dendrimer were put into the quartz cells, and the time-courses of the absorption changes from the solutions were monitored (Fig. 3). In the absence of G2 POSS-core dendrimer, the absorption decreased, and the magnitude of the absorption decreased by 50% after 7 days. In addition, the quartz cell was obviously stained. These results indicate that TP3PY should be adsorbed onto the wall of the vessels. On the other hand, the significant decrease was hardly observed in the presence of G2 POSS-core dendrimer even after 7 days. The absorption was maintained at 94%. Moreover, the quartz cell seemed to be kept clean after the experiments. These results clearly indicate that G2 POSS-core dendrimer has the superior ability to improve the stability of the dye against undesired aggregation or adsorption in PBS by encapsulating.

The emission properties of TP3PY in the presence or absence of G2 POSS-core dendrimer were investigated in various solvents (Fig. 4). Significant enhancements of quantum yields were observed by the encapsulation in each solvent (Table 1). The quantum yield of the emission of TP3PY was enhanced 6-folds by encapsulating into the dendrimers in THF due to the increase of the magnitude of emission intensity. In water and acetonitrile, new emission bands were observed. From these data, it is proposed that G2 POSS-core dendrimer plays a significant role in the improvements of the emission intensity of TP3PY. As shown in Table 1, the peak position of TP3PY was strongly affected by the solvent polarity. Nevertheless, the peak positions of the emission were slightly shifted before and after the complexation. These data suggest that the polarity changes caused by the complex formation should be hardly responsible for the enhancement of the optical properties.

To clarify the mechanism of the enhancement to the quantum yields by encapsulating into the POSS-core dendrimer, the series of analyses were executed. According to the solvatochromic shift of the emission from TP3PY, the Lippert–Mataga plot (Fig. S1) is prepared.[14] The linear relationship between the solvent polarity and the Stokes shift was obtained. This fact indicates that the emission band with the peak at 650 nm of TP3PY is assigned as the induced charge transfer band. In addition, the transition dipole moment ($\Delta\mu$) was calculated as 11.80 D from the slope of the fitting line. The relatively larger $\Delta\mu$ value proposes that the emission of TP3PY at 650 nm is assigned as a twisted-intramolecular charge transfer (TICT) band.[5,14] The new emission peak at 550 nm assigned as the localized excitation (LE) band was observedat −196 °C in 2-methyltetrahydrofuran, resulting in the dual-emission (Fig. 5). These data also support the assumption that the emission of TP3PY is originated from the TICT band. From the data, it can be claimed that the POSS-core dendrimer has the significant effect to suppress the motions of the encapsulated molecules. We measured the fluorescence life times of TP3PY (Fig. 6). Interestingly, it was revealed that the new emission with the peak at 550 nm had a long life time. It is suggested that the emission from the LE band appeared after the encapsulation. The twisting at the excited state was inhibited by encapsulating into the POSS-core dendrimers at room temperature. These results clearly indicate that the non-irradiation decay caused by the molecular tumbling should be significantly suppressed by encapsulating into the POSS-core dendrimer, and therefore, the emission of TP3PY could be enhanced. Moreover, the optical probes having the longer life

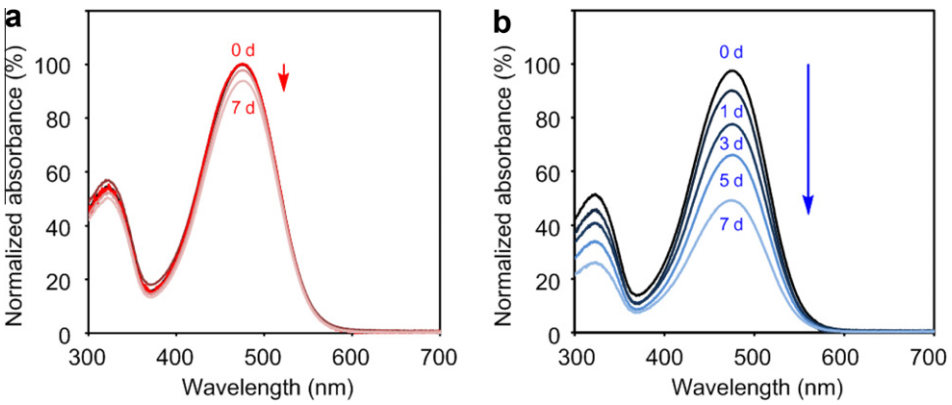

Figure 3. Time-courses of the absorption changes of TP3PY in the filtrates in the presence (a) or absence (b) of G2 POSS-core dendrimer (10 equiv). The samples in PBS were stored at 25 °C in darkness and passed through the size-exclusive filter.

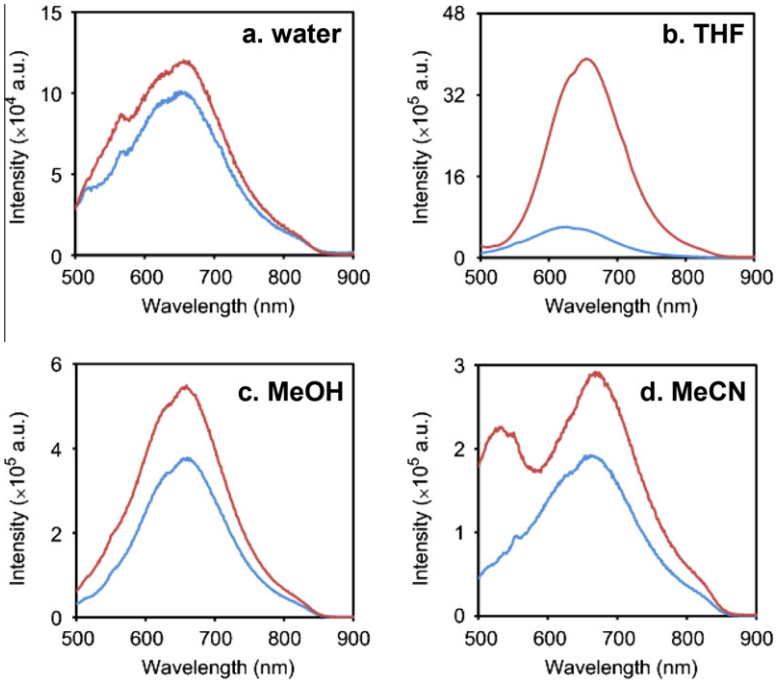

Figure 4. Emission spectra of TP3PY in the presence (red line) or absence (blue line) of G2 POSS-core dendrimer (10 equiv) at 25 °C in various kinds of solvents with the excitation light at 474 nm. The stock solutions of TP3PY (×10) and G2 POSS-core dendrimer (×10) were mixed at room temperature, and then 500 μL of the samples were prepared by adding the solvents. The measurements were executed within 10 min.

Table 1
Optical properties of TP3PY[a]

	TP3PY			Dendrimer complex[b]		
	λ_{em}[c] (nm)	$\Delta\lambda$[d] (nm)	Φ[e] (×10^{-2})	λ_{em}[c] (nm)	$\Delta\lambda$[d] (nm)	Φ[e] (×10^{-2})
THF	621	147	0.658	634	160	4.02
MeCN	648	174	0.320	649	175	0.390
MeOH	646	172	0.566	646	172	0.687
Water	648	174	0.267	655	181	0.343

[a] 10 μM solutions.
[b] In the presence of 100 μM G2 POSS-core dendrimer.
[c] Excitation wavelength was at 474 nm.
[d] Stokes shifts.
[e] Determined as a relative value.

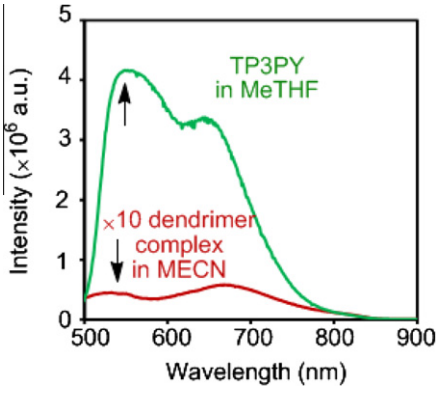

Figure 5. Emission spectra of TP3PY at −196 °C in 2-methyltetrahydrofuran with the excitation light at 474 nm.

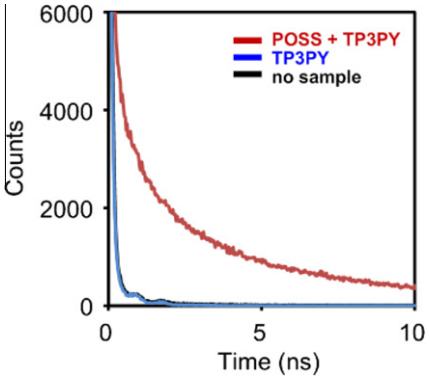

Figure 6. Fluorescence decay curves of TP3PY in PBS. All measurements were monitored at 25 °C with the excitation light at 375 nm.

times were feasible for improving the signal to noise ratio by delaying the detection time for subtracting the self-emission. The encapsulation by the POSS-core dendrimer can be applied for this purpose.

4. Conclusion

We describe that the POSS-core dendrimer enhanced various kinds of the properties of TP3PY for applying a bioprobe. Initially,

the TP3PY complexes with G2 POSS-core dendrimer showed superior dispersibility in the buffer. The undesired adsorption of TP3PY onto the vessels was effectively suppressed. In addition, the improvement of quantum yields and elongation of fluorescent lifetime were observed from the dendrimer/dye complexes. It was proposed that the molecular rotation of TP3PY at the excited state could be suppressed in the complexes with the POSS-core dendrimer. Consequently, the emission band which is detected at low temperature can be observed at room temperature from the dendrimer/dye complexes. These results represent that the POSS-core dendrimers can create the special spots where the molecular motion of the entrapped molecules should significantly decrease in water.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.055.

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