

Enhancement of Entrapping Ability by Cubic Silsesquioxane Core in Dendrimers

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We report that the POSS core can enhance the entrapping ability of the dendrimer. Compared to the G2 PAMAM dendrimer, the G2 POSS-core dendrimer can entrap a larger amount of guest molecules without loss of affinity, and consequently the water-solubility of the entrapped guest molecules can be increased. In addition, we demonstrate that the entrapped fluorophore into the G2 POSS-core dendrimer was prevented from the fluorescence photobleaching.

Water-soluble dendrimers have been used as the convenient vehicles for drug delivery not only due to the enhancement of water-solubility to the hydrophobic molecules by the packing into the internal space but also due to the site-selective distribution by the size tuning and the peripheral modification. Poly(amidoamine) (PAMAM) dendrimers, which are well known as the typical water-soluble dendrimers, have been proposed as mimics of charged micelles or proteins because of their unimolecular characters, and their physicochemical properties and biological behaviors have been investigated extensively.^[1]

The inside of dendrimers can generate the distinctive space in the solution. The different polarity, solvation, and structure can provide dendrimers with the characteristics as the reaction fields, the molecular gates, and the templates for the synthesis of nanoparticles.^[2-4] The core of the dendrimers plays a crucial role in these properties via the predominance on the total shape and the groove between dendrons, particularly in the early generation. From this view point, the polyhedral structure of the polyhedral oligomeric silsesquioxane (POSS) core is very attractive because the internal space of POSS-core dendrimers has a possibility to contribute to generate new properties because of their three-dimensional architecture (Fig. 1).^[5]

Herein, we report that the POSS core can enhance the entrapping ability to the dendrimers in aqueous media. Compared to the G2 PAMAM dendrimer, the G2 POSS-core dendrimer can capture a larger amount of guest molecules without loss of the affinity, and consequently the water-solubility of the guest molecules can be increased. In addition, we demonstrate the photochemical

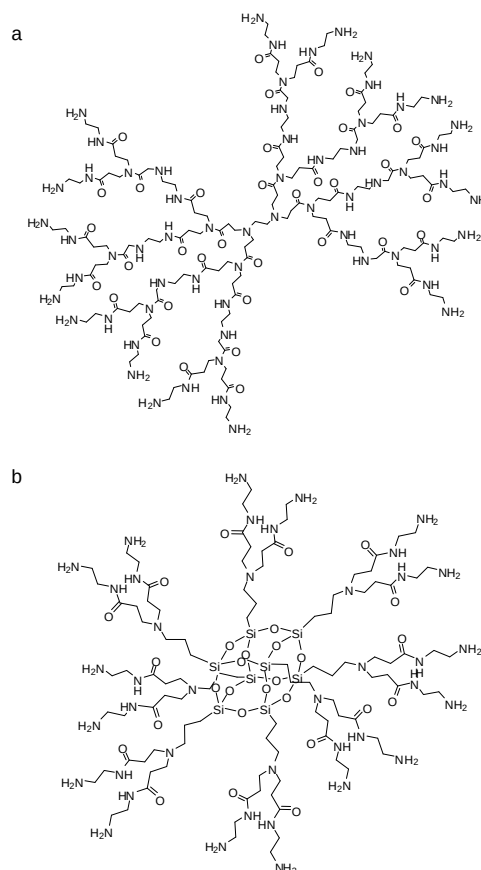


Fig. 1 Chemical structures of (a) the G2 PAMAM dendrimer and (b) the G2 POSS-core dendrimer.

application to prevent the entrapped fluorophore from the fluorescence photobleaching.

Previous reports suggested that POSS-core dendrimers have a relatively globular conformation and few entanglements of their branches with a high proportion of terminal functional groups positioned on the external surface of the dendrimers even in earlier generations.^[6] In contrast, the early generation PAMAM dendrimers can form an open structure.^[7] Therefore, we expected that the difference of the core between the G2 PAMAM and POSS-core dendrimer should influence on the quantity, the universality, and the affinity with the G2 POSS-core dendrimer in the entrapment of the guest molecules.

In order to evaluate the entrapping ability of each dendrimer, the enhancement solubilization factor (ESF) defined as the number of moles of compound solubilized per number of moles of the

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Table 1 The enhancement solubilization factor (ESF) and the dissociation temperature (T_d) for the polycyclic aromatic compounds

Dendrimers	Anthracene		Naphthacene		Phenanthrene		Pyrene	
	ESF	T_d (°C) ^a	ESF	T_d (°C) ^a	ESF	T_d (°C) ^a	ESF	T_d (°C) ^a
G2 POSS-core	0.6	47.3	1.2	>80 ^b	1.4	62.5	0.8	46.5
G2 PAMAM	0.6	49.0	1.2	>80 ^b	0.1	53.1	0.5	47.0

^aAll T_d s of the complexes (10 μ M) were taken in 50 mM sodium phosphate buffers (pH = 7.0). First derivatives were calculated to determine T_d values.

^b T_d s were not determined because of too high affinity.

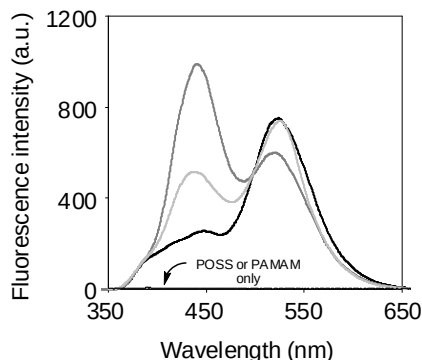


Fig. 2 Fluorescence spectra of 1 μ M DAN in the absence (black line) and presence of 10 μ M the dendrimers (G2 POSS-core dendrimer: dark gray line, G2 PAMAM dendrimer: light gray line) in 50 mM sodium phosphate buffer (pH = 7.0) at 25 °C. Excitation wavelength was 300 nm.

dendrimers was evaluated with the G2 PAMAM and POSS-core dendrimer in 50 mM sodium phosphate buffer (pH = 7.0) at 25 °C.^[8] Samples containing the guest molecules and each dendrimer were sonicated for 30 sec and allowed to equilibrate in darkness overnight for the complexation with the guest molecules. The ESF values were calculated from the difference between the solubility of the guest molecules in the presence and absence of dendrimers with UV absorption spectra.^[9] The results are summarized in Table 1. The G2 POSS-core dendrimer can capture the larger amounts of the planner molecules, phenanthrene and pyrene, than those of the G2 PAMAM dendrimer, while similar amounts of the linear molecules, anthracene and naphthacene were entrapped into both dendrimers. The globular structure of the POSS-core dendrimer could generate the hydrophobic cavity for entrapping the planner molecules.

Affinity of entrapped molecules with dendrimers was estimated by the dissociation temperature (T_d) obtained from variable temperature UV measurements (Table 1).^[10] Each guest molecule showed different UV absorbance between inside and outside dendrimers. We decided the T_d values between the guest molecules and the dendrimers from the chromism in the UV spectra. Except the complex with naphthacene, the traces of the absorbance alteration of aromatic rings in the sample solutions exhibited the sigmoid curves, and the T_d values were determined from the temperatures at the flexion points on the curves. The affinities with anthracene, naphthacene, and pyrene were not significantly influenced by the POSS-core substitution. Large stabilization was observed even in the complex with phenanthrene which was hardly captured by the G2 PAMAM dendrimer. Including of the result of the ESF measurements, these data suggest that the water-exclusive space and less entanglement of dendrons around the POSS core could produce the favorable pockets for molecular capturing.

For investigating the heterogeneous environments of the dendrimers by the photochemical approach, we used 6-dimethylamino-2-naphthaldehyde (DAN) known as a micro-

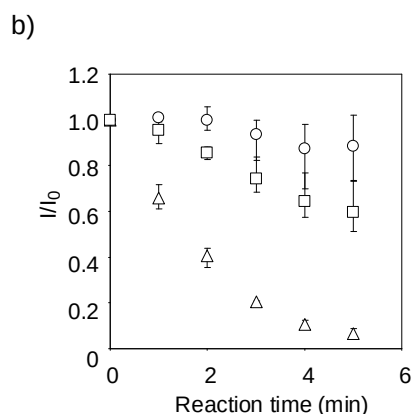
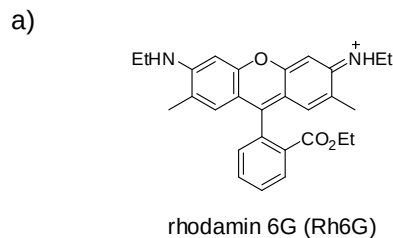


Fig. 3 (a) Chemical structure of Rh6G. (b) Time-course of the decrease of the fluorescence intensity of Rh6G (1 μ M) (triangular dots) in aerated water in the presence of 10 μ M G2 POSS-core dendrimer (circular dots) or G2 PAMAM dendrimer (square dots) followed through the decrease of the fluorescence emission with increasing time of UV irradiation with a low pressure mercury lamp at 25 °C. The data points represent the average of three sets of independent experiments, and error bars represent standard deviation.

environment-sensitive fluorescent probe.^[11] All DAN molecules in the solution were entrapped into the excess of the dendrimers. The sample containing 1 μ M DAN in 50 mM sodium phosphate buffer (pH = 7.0) excited at 300 nm wavelength gave fluorescence emission at 525 nm (Fig. 2). By the complexation with both of the dendrimers (10 μ M), the new peak of fluorescence emission appeared at 440 nm. In particular, the fluorescence spectra of the complex with the G2 POSS-core dendrimer showed the significant change from that of the sample without dendrimer. These data suggest that the G2 POSS-core dendrimer could make stronger interaction with the guest molecules than the G2 PAMAM dendrimer, and it is implied that this interaction could contribute to the enhancement of the amount of the guest molecules encapsulated by the G2 POSS-core dendrimer.

For the repetitive and longitudinal measurements with microscopy or time-resolve spectroscopy, efforts have been made to improve the fluorescence of the dyes, in regard to their stability towards adsorption, aggregation, and photochemical decomposition, by use of additives.^[12-15] We demonstrate the prevention of fluorescence photobleaching of rhodamine 6G (Rh6G), which is the most important fluorescent dye as shown by the classical and

contemporary applications, by the entrapment with POSS-core dendrimers (Fig. 3a).^[12,16] Though the G2 PAMAM dendrimer showed less interaction with Rh6G^[17], the G2 POSS-core dendrimer can efficiently capture Rh6G without changing the fluorescence spectra of Rh6G after the complexation. The fluorescence intensity was monitored after UV irradiation with the low pressure mercury lamp at 25 °C. The fluorescence emission obtained from the aqueous solution containing 1 μ M Rh6G in 50 mM sodium phosphate buffer (pH = 7.0) was greatly reduced to 10% after 5 min UV irradiation (Fig. 3b). In the presence of 10 μ M G2 PAMAM dendrimer, the fluorescence emission of Rh6G was reduced to 60% after irradiation. Markedly, the fluorescence emission from the sample containing the G2 POSS-core dendrimer remained approximately 90% after 5 min irradiation. This significant advantage of the entrapment into POSS-core dendrimers to suppress the optical degradation should be valuable for the experimental usages of common imaging probes as well as fluorescence dyes.

In conclusion, we described here that the POSS core can enhance the entrapping ability to the dendrimers. Compared to the G2 PAMAM dendrimer, a larger amount of guest molecules such as hydrophobic aromatic rings or fluorescence dyes can be captured by the G2 POSS-core dendrimers. In addition, effective inhibition from the fluorescence photobleaching of the entrapped molecules was accomplished. Though there remains room to investigate the toxicity and the releasing ability of the POSS-core dendrimers for practical usage as the carrier in drug delivery or in vivo imaging, this work suggests the potential widespread application of POSS-core dendrimers not only for medicinal science but also for biotechnology.

Notes and references

- 1 R. Esfand, D. A. Tomalia, *Drug Discov. Today*, 2001, **6**, 427; D. A. Tomalia, L. A. Reyna, S. Svenson, *Biochem. Soc. T.*, 2007, **35**, 61; X. Shi, I. J. Majoros, J. R. Baker, *Mol. Pharm.*, 2005, **2**, 278; C. Kojima, K. Kono, K. Maruyama, T. Takagishi, *Bioconjugate Chem.*, 2000, **11**, 910.
- 2 R. van Heerbeek, P. C. J. Kamer, P. W. N. M. van Leeuwen, J. N. H. Reek, *Chem. Rev.*, 2002, **102**, 3717; S. M. Grayson, J. M. J. Fréchet, *Chem. Rev.*, 2001, **101**, 3819; D. Astruc, F. Chardac, *Chem. Rev.*, 2001, **101**, 2991; T. Mizugaki, M. Murata, S. Fukubayashi, T. Mitsudome, K. Jitsukawa, K. Kaneda, *Chem. Commun.*, 2008, 241; M. A. Castriciano, A. Romeo, M. C. Baratto, R. Pogni, L. M. Scolaro, *Chem. Commun.*, 2008, 688.
- 3 J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Science*, 1994, **266**, 1226; H. Martin, H. Kinns, N. Mitchell, Y. Astier, R. Madathil, S. Howorka, *J. Am. Chem. Soc.*, 2007, **129**, 9640; J. F. G. A. Jansen, E. W. Meijer, *J. Am. Chem. Soc.*, 1995, **117**, 4417; S. Xu, Y. Luo, R. Haag, *Macromol. Biosci.*, 2007, **7**, 968.
- 4 N. Satoh, T. Nakashima, K. Kamikura, K. Yamamoto, *Nat. Nanotechnol.*, 2008, **3**, 106; O. Varnavski, R. G. Ispasoiu, L. Balogh, D. Tomalia, T. Goodson III, *J. Chem. Phys.*, 2001, **114**, 1962; R. W. J. Scott, H. Ye, R. R. Henriquez, R. M. Crooks, *Chem. Matter.*, 2003, **15**, 3873; R. W. J. Scott, O. M. Wilson, R. M. Crooks, *J. Phys. Chem. B*, 2005, **109**, 692; H. Lang, R. A. May, B. L. Iversen, B. D. Chandler, *J. Am. Chem. Soc.*, 2003, **125**, 14832; D. A. Tomalia, A. M. Naylor, W. A. Goddard III, *Angew. Chem. Int. Edn. Engl.*, 1990, **29**, 138.
- 5 K. Naka, M. Fujita, K. Tanaka, Y. Chujo, *Langmuir*, 2007, **23**, 9057.
- 6 P.-A. Jaffrés, R. E. Morris, *J. Chem. Soc., Dalton Trans.*, 1998, 2767; F. J. Feher, K. D. Wyndham, *Chem. Commun.*, 1998, 323; F. J. Feher, K. D. Wyndham, D. Soulivong, F. Nguyen, *J. Chem. Soc., Dalton Trans.*, 1999, 1491; X. Zhang, K. J. Haxton, L. Ropartz, D. J. Cole-Hamilton, R. E. Morris, *J. Chem. Soc., Dalton Trans.*, 2001, 3261.

- 7 G. Caminati, N. J. Turro, D. A. Tomalia, *J. Am. Chem. Soc.*, 1990, **112**, 8515.
- 8 L. Fernandez, M. Gonzalez, H. Cerecetto, M. Santo, J. J. Silber, *Supramol. Chem.*, 2006, **18**, 633.
- 9 See Figure S1 in the Supporting Information.
- 10 See Figure S2 in the Supporting Information.
- 11 G. Weber, F. J. Farris, *Biochemistry*, 1979, **18**, 3075; R. B. MacGregor, G. Weber, *Ann. N.Y. Acad. Sci.*, 1981, **366**, 140; F. G. Prendergast, M. Meyer, G. L. Carlson, S. Iida, J. D. Potter, *J. Biol. Chem.*, 1983, **258**, 7541; R. B. MacGregor, G. Weber, *Nature*, 1986, **319**, 70-73; K. Tainaka, K. Tanaka, S. Ikeda, K. Nishiza, T. Unzai, Y. Fujiwara, I. Saito, A. Okamoto, *J. Am. Chem. Soc.*, 2007, **129**, 4776.
- 12 C. Eggeling, J. Widengren, R. Rigler, C. A. M. Seidel, in *Applied Fluorescence in Chemistry, Biology and Medicine* (Eds.: W. Rettig, B. Strehmel, S. Schrader, H. Seifert), Springer, Heidelberg, 1999, pp.193.
- 13 E. Arunkumar, C. C. Forbes, B. D. Smith, *Eur. J. Org. Chem.*, 2005, 4051.
- 14 J. Mohanty, W. M. Nau, *Angew. Chem. Int. Ed.*, 2005, **44**, 3750.
- 15 J. C. Mialocq, M. Meyer, P. Hébert, X. Armand, D. Lambert, *Opt. Commun.*, 1990, **77**, 185.
- 16 C. Eggeling, J. Widengren, R. Rigler, C. A. M. Seidel, *Anal. Chem.*, 1998, **70**, 2651.
- 17 Entrapping by the G2 PAMAM dendrimer was not confirmed because of less influence on UV spectra of Rh6G by the entrapping of the G2 PAMAM dendrimer. See Figure S4 in the Supporting Information.