Enhancement of entrapping ability of dendrimers by a cubic silsesquioxane core†

Kazuo Tanaka,[a] Kenichi Inafuku,[a] Kensuke Naka[b] and Yoshiki Chujo*[a]

Received 18th July 2008, Accepted 22nd September 2008
First published as an Advance Article on the web 29th September 2008
DOI: 10.1039/b812349g

We report that a polyhedral oligomeric silsesquioxane (POSS) core can enhance the entrapping ability of dendrimers. 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 demonstrated that a fluorophore entrapped in the G2 POSS-core dendrimer was prevented from undergoing fluorescence photobleaching.

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

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

Herein, we report that the POSS core can enhance the entrapping ability of 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 a photochemical application to prevent the entrapped fluorophore from undergoing fluorescence photobleaching.

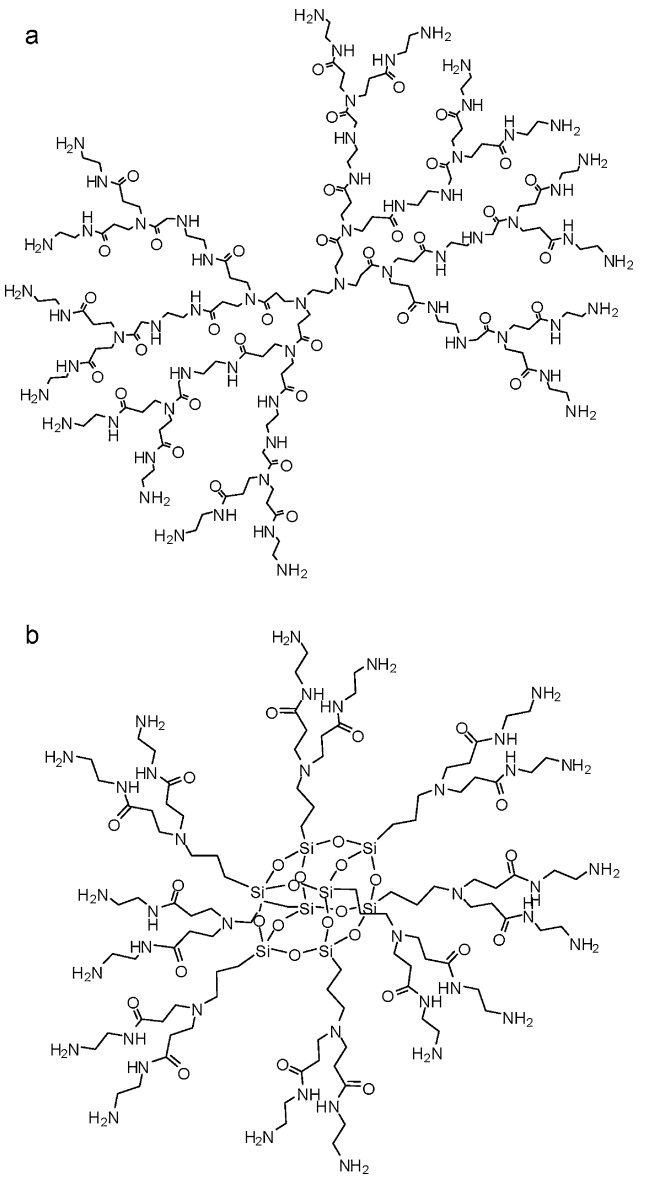

Fig. 1 Chemical structures of (a) the G2 PAMAM dendrimer and (b) the G2 POSS-core dendrimer.

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 in the core between the G2 PAMAM and

[a]*Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan. E-mail: chujo@chujo.synchem.kyoto-u.ac.jp; Fax: (+81) 75-383-2605*
[b]*Department of Chemistry and Materials Technology, Graduate School of Science and Technology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan*

† Electronic supplementary information (ESI) available: Experimental and Fig. S1–S4. See DOI: 10.1039/b812349g

POSS-core dendrimer should influence the quantity, the universality, and 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 dendrimers, was evaluated with the G2 PAMAM and POSS-core dendrimers 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 seconds and allowed to equilibrate in darkness overnight for 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 larger amounts of the planar molecules, phenanthrene and pyrene, than those of the G2 PAMAM dendrimer, while similar amounts of the linear molecules, anthracene and naphthacene were entrapped in both dendrimers. The globular structure of the POSS-core dendrimer could generate a hydrophobic cavity for entrapping the planar molecules.

The 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 the 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 for the complex with naphthacene, the traces of the absorbance alteration of aromatic rings in the sample solutions exhibited 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 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 favorable pockets for molecular capturing.

For investigating the heterogeneous environments of the dendrimers by a photochemical approach, we used 6-dimethylamino-2-naphthaldehyde (DAN), known as a micro-environment-sensitive fluorescent probe.[11] All DAN molecules in the solution were entrapped into an excess of the dendrimers. The sample containing 1 μM DAN in 50 mM sodium phosphate buffer (pH = 7.0), excited at 300 nm wavelength, gave fluorescence emission at 525 nm (Fig. 2). By complexation with both of the dendrimers (10 μM), the new peak of fluorescence emission appeared at 440 nm. In particular, the fluorescence spectra of the complex

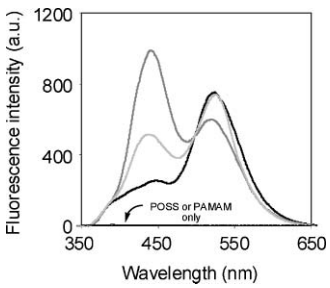

Fig. 2 Fluorescence spectra of 1 μM DAN in the absence (black line) and presence of 10 μM of the dendrimers (G2 POSS-core dendrimer: dark grey line, G2 PAMAM dendrimer: light grey line) in 50 mM sodium phosphate buffer (pH = 7.0) at 25 °C. The excitation wavelength was 300 nm.

with the G2 POSS-core dendrimer showed a significant change from that of the sample without dendrimer. These data suggest that the G2 POSS-core dendrimer could interact more strongly 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 guest molecules encaptured by the G2 POSS-core dendrimer.

For the repetitive and longitudinal measurements with microscopy or time-resolved 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 classical and contemporary applications, by 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 complexation. The fluorescence intensity was monitored after UV irradiation with a low pressure mercury lamp at 25 °C. The fluorescence emission obtained from the aqueous solution containing 1 μM 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 μM 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 at approximately 90% after 5 min irradiation. This significant advantage of entrapment into POSS-core dendrimers to suppress 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 of dendrimers. Compared to the G2

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

	Anthracene		Naphthacene		Phenanthrene		Pyrene	
Dendrimers	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

[a] All T_ds of the complexes (10 μM) were taken in 50 mM sodium phosphate buffers (pH = 7.0). First derivatives were calculated to determine T_d values. [b] T_ds were not determined because of a too high affinity.

 This journal is © The Royal Society of Chemistry 2008

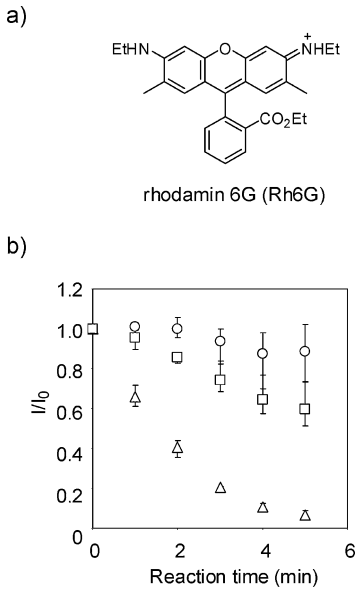

Fig. 3 (a) Chemical structure of Rh6G. (b) Time-course of the decrease of the fluorescence intensity of Rh6G (1 μM) (triangular dots) in aerated water in the presence of 10 μM G2 POSS-core dendrimer (circular dots) or G2 PAMAM dendrimer (square dots) followed through the decrease of the fluorescence 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.

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 of 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 a 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 and D. A. Tomalia, *Drug Discovery Today*, 2001, **6**, 427; D. A. Tomalia, L. A. Reyna and S. Svenson, *Biochem. Soc. Trans.*, 2007, **35**, 61; X. Shi, I. J. Majoros and J. R. Baker, *Mol. Pharmacol.*, 2005, **2**, 278; C. Kojima, K. Kono, K. Maruyama and T. Takagishi, *Bioconjugate Chem.*, 2000, **11**, 910.

2 R. van Heerbeek, P. C. J. Kamer, P. W. N. M. van Leeuwen and J. N. H. Reek, *Chem. Rev.*, 2002, **102**, 3717; S. M. Grayson and J. M. J. Fréchet, *Chem. Rev.*, 2001, **101**, 3819; D. Astruc and F. Chardac, *Chem. Rev.*, 2001, **101**, 2991; T. Mizugaki, M. Murata, S. Fukubayashi, T. Mitsudome, K. Jitsukawa and K. Kaneda, *Chem. Commun.*, 2008, 241; M. A. Castriciano, A. Romeo, M. C. Baratto, R. Pogni and L. M. Scolaro, *Chem. Commun.*, 2008, 688.

3 J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg and E. W. Meijer, *Science*, 1994, **266**, 1226; H. Martin, H. Kinns, N. Mitchell, Y. Astier, R. Madathil and S. Howorka, *J. Am. Chem. Soc.*, 2007, **129**, 9640; J. F. G. A. Jansen and E. W. Meijer, *J. Am. Chem. Soc.*, 1995, **117**, 4417; S. Xu, Y. Luo and R. Haag, *Macromol. Biosci.*, 2007, **7**, 968.

4 N. Satoh, T. Nakashima, K. Kamikura and K. Yamamoto, *Nat. Nanotechnol.*, 2008, **3**, 106; O. Varnavski, R. G. Ispasoiu, L. Balogh, D. Tomalia and T. Goodson III, *J. Chem. Phys.*, 2001, **114**, 1962; R. W. J. Scott, H. Ye, R. R. Henriquez and R. M. Crooks, *Chem. Mater.*, 2003, **15**, 3873; R. W. J. Scott, O. M. Wilson and R. M. Crooks, *J. Phys. Chem. B*, 2005, **109**, 692; H. Lang, R. A. May, B. L. Iversen and B. D. Chandler, *J. Am. Chem. Soc.*, 2003, **125**, 14832; D. A. Tomalia, A. M. Naylor and W. A. Goddard III, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 138.

5 K. Naka, M. Fujita, K. Tanaka and Y. Chujo, *Langmuir*, 2007, **23**, 9057.

6 P.-A. Jaffrés and R. E. Morris, *J. Chem. Soc., Dalton Trans.*, 1998, 2767; F. J. Feher and K. D. Wyndham, *Chem. Commun.*, 1998, 323; F. J. Feher, K. D. Wyndham, D. Soulivong and F. Nguyen, *J. Chem. Soc., Dalton Trans.*, 1999, 1491; X. Zhang, K. J. Haxton, L. Ropartz, D. J. Cole-Hamilton and R. E. Morris, *J. Chem. Soc., Dalton Trans.*, 2001, 3261.

7 G. Caminati, N. J. Turro and D. A. Tomalia, *J. Am. Chem. Soc.*, 1990, **112**, 8515.

8 L. Fernandez, M. Gonzalez, H. Cerecetto, M. Santo and J. J. Silber, *Supramol. Chem.*, 2006, **18**, 633.

9 See Fig. S1 in the supporting information.

10 See Fig. S2 in the supporting information.

11 G. Weber and F. J. Farris, *Biochemistry*, 1979, **18**, 3075; R. B. MacGregor and G. Weber, *Ann. N. Y. Acad. Sci.*, 1981, **366**, 140; F. G. Prendergast, M. Meyer, G. L. Carlson, S. Iida and J. D. Potter, *J. Biol. Chem.*, 1983, **258**, 7541; R. B. MacGregor and G. Weber, *Nature*, 1986, **319**, 70–73; K. Tainaka, K. Tanaka, S. Ikeda, K. Nishiza, T. Unzai, Y. Fujiwara, I. Saito and 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*, ed. W. Rettig, B. Strehmel, S. Schrader and H. Seifert, Springer, Heidelberg, 1999, p. 193.

13 E. Arunkumar, C. C. Forbes and B. D. Smith, *Eur. J. Org. Chem.*, 2005, 4051.

14 J. Mohanty and W. M. Nau, *Angew. Chem., Int. Ed.*, 2005, **44**, 3750.

15 J. C. Mialocq, M. Meyer, P. Hébert, X. Armand and D. Lambert, *Opt. Commun.*, 1990, **77**, 185.

16 C. Eggeling, J. Widengren, R. Rigler and C. A. M. Seidel, *Anal. Chem.*, 1998, **70**, 2651.

17 Entrapping by the G2 PAMAM dendrimer was not confirmed because there was less influence on the UV spectra of Rh6G by entrapping the G2 PAMAM dendrimer. See Fig. S4 in the supporting information.