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Enhancement of Entrapping Ability by Cubic Silsesquioxane Core in Dendrimers

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

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(amideamine) (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.[2–5] 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 viewpoint, 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).[6]

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 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

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

[1] Department of Polymer Chemistry
Graduate School of Engineering, Kyoto University
Katsura, Nishikyo-ku, Kyoto 615-8510 Japan
Fax: (+81) 75-383-2605
E-mail: chujo@chujo.synchem.kyoto-u.ac.jp
[2] Department of Chemistry and Materials Technology
Graduate School of Science and Technology
Kyoto Institute of Technology
Sakyo-ku, Kyoto 606-8585 Japan
[3] Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See http://dx.doi.org/10.1039/b000000x/
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 naphthalene 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 naphthalene, 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, naphthalene, 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-
environment-sensitive fluorescent probe.[11] All DAN molecules in
the solution were entrapped into the 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 the 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 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 encaptured
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

<table>
<thead>
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<th>Dendrimers</th>
<th>Anthracene ESF</th>
<th>Naphthalene ESF</th>
<th>Phenanthrene ESF</th>
<th>Pyrene ESF</th>
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</thead>
<tbody>
<tr>
<td>G2 POSS-core</td>
<td>0.6</td>
<td>1.2</td>
<td>&gt;80°</td>
<td>1.4</td>
</tr>
<tr>
<td>G2 PAMAM</td>
<td>0.6</td>
<td>1.2</td>
<td>&gt;80°</td>
<td>0.1</td>
</tr>
</tbody>
</table>

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

\[ \text{Anthracene, Naphthalene, Phenanthrene, and Pyrene were not significantly altered in the } \text{complex with naphthalene, the trace} \]

Fig. 2 Fluorescence spectra of 1 μM DAN in the absence (black line) and presence of 10 μM 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.

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 (circles) 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.
contemporary applications, by the entrapment with POSS-core dendrimers (Fig. 3a).[12,13] 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 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 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