Control of Temperature-Sensitive Properties of Poly(amidoamine) Dendrimers Using Peripheral Modification with Various Alkylamide Groups

Yasuhiro Haba, Chie Kojima, Atsushi Harada, and Kenji Kono*

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Nakaku, Sakai, Osaka 599-8531, Japan

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Introduction

Dendrimers have various features that conventional linear polymers do not have.[1] Their size, structure, and surface properties are highly controllable. In addition, their interiors can encapsulate small molecules. Using these features of dendrimers, materials with unique and important functions are expected to be produced. Stimuli-sensitive properties are useful functions considering application of dendrimers in the fields of drug delivery[2] and catalysis.[3] Therefore, many efforts have been made to develop dendrimers with sensitivity to light[4] and oxdative−reductive environments.[5]

Regarding the preparation of dendrimers with temperature sensitivity, attempts have been made to graft poly(*N*-isopropylacrylamide) (PNIPAM), which is a well-known thermosensitive polymer with a lower critical solution temperature (LCST) of 32 °C, to chain ends of dendrimers.[6,7] Indeed, attachment of PNIPAM chains to the dendrimer surface produces dendrimers with temperature-sensitive shells. However, it is noteworthy that these PNIPAM-modified dendrimers lose their important characteristic of molecular uniformity because PNIPAM chains are fundamentally polydispersed. In addition, the molecular shape of the PNIPAM-modified dendrimers might more closely resemble that of star polymers rather than globular dendrimers.

Recently, we presented another strategy for preparation of thermosensitive dendrimers. We introduced isobutyramide (IBAM) group, a common structural unit with thermosensitive poly(*N*-vinylisobutyramide), to every chain end of dendrimers.[8] We observed that the IBAM-terminated poly(amidoamine) or poly(propylenimine) dendrimer exhibited lower critical solution temperature. This strategy is attractive because the resultant dendrimers with temperature-sensitive properties retain their globular structure and uniformity at the molecular level.

On the basis of this design of IBAM-modified dendrimers, a question of whether temperature-sensitive dendrimers are obtainable using surface modification with structural units common with thermosensitive polymers naturally arises. To address this question, we prepared PAMAM dendrimers in this study that have various alkylamide groups, which share structural similarity with thermosensitive polymers with linear structure.[9,10] The correlation between the structures of alkylamide groups in the dendrimer periphery and their temperature-sensitive properties has been described.

Results and Discussion

Previous studies have shown that various molecules that have a carboxyl group, such as isobutyric acid and *tert*-butyloxycarbonyl-protected amino acids, can be combined to every chain end of amine-terminated PAMAM dendrimers using the condensing agent 1,3-dicyclohexylcarbodiimide (DCC).[5,8,11] Therefore, in the same manner, we prepared PAMAM G4 dendrimers that have *n*-butyramide (NBAM), IBAM, and cyclopropanecarboxylic acid amide (CPCAM) groups at chain ends, which are designated as NBAM-G4, IBAM-G4, and CPCAM-G4, by the reaction of amine-terminated PAMAM G4 dendrimer with *n*-butyric acid, isobutyric acid, and cyclopropanecarboxylic acid using DCC (Scheme 1). Furthermore, PAMAM G5 dendrimers that have propionamide (PAM), NBAM, and *n*-valeramide (VAM) groups at chain ends, which are designated as PAM-G5, NBAM-G5, and VAM-G5, were synthesized using the reaction of amine-terminated PAMAM G5 dendrimer with the corresponding carboxylic acids in the same way. The numbers of alkylamide groups of the alkylamide-terminated dendrimers were evaluated using ^{1}H NMR (Table l). As shown in Table 1, the numbers of terminal alkylamide groups agree well with the numbers of the chain ends of the corresponding dendrimers for all alkylamide-modified dendrimers, indicating that essentially every chain end of the dendrimer is combined to the alkylamide group for these modified dendrimers.

First, we investigated the influence of the length of the terminal alkylamide groups on thermosensitivity of the alkylamide-terminated dendrimers. Figure 1 portrays the temperature dependence of the light transmittance of PAM-G5, NBAM-G5, and VAM-G5 dissolved in 10 mM phosphate solution of pH 9.0 at 500 nm. The solution of the PAM-G5 dendrimer was transparent over the experimental temperature range. However, the solutions of NBAM-G5 and VAM-G5 dendrimers became turbid at 43 and 11 °C, respectively, indicating that these dendrimer solutions underwent phase separation at these temperatures. In general, the LCST of thermosensitive polymers is known to decrease with increasing hydrophobicity of the polymer chains.[9,10,12] Regarding poly(*N*-vinylalkylamide)s, which are polymers with a linear structure that shares common structural units with these alkylamide-terminated dendrimers, the LCST of poly(*N*-vinyl-*n*-butyramide) is reported as 32 °C. In contrast, poly(*N*-vinyl-*n*-valeramide) is insoluble in water, even in cold water, because of its hydrophobic side groups.[9] Therefore, it is likely that PAM groups are too hydrophilic to provide thermosensitive properties to the PAMAM G5 dendrimer, whereas NBAM and VAM groups might have appropriate hydrophobicity to yield dendrimer with temperature sensitivity.

Next, for alkylamide-terminated dendrimers, we investigated the influence of structures of terminal alkylamide groups that have the same number of carbon atoms on their thermosensitive properties. Figure 2 shows the temperature dependence of the light transmittance of NBAM-G4, IBAM-G4, and CPCAM-G4 dissolved in 10 mM phosphate solution of pH 9.0 at 500 nm. Solutions of NBAM-G4, IBAM-G4, and CPCAM-G4 exhibited the cloud points at 45, 61, and 15 °C, respectively.

Hydrophobicity of alkyl group with a branch or cyclic structure is known to be lower than for the alkyl group of the same number of carbon atoms with a linear structure. We calculated logarithms of 1-octanol−water partition coefficient (log *P*), which are used to estimate hydrophobicity of a molecule, for *N*-ethylbutyramide, *N*-ethylisobutyramide, and cyclopropanecarboxylic acid ethylamide, which bear structural similarity to the terminal moiety of the alkylamide-modified

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* Corresponding author: Tel and Fax: +81-72-254-9330; e-mail: kono@chem.osakafu-u.ac.jp.

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Scheme 1. Synthesis of PAMAM G4 and G5 Dendrimers Having Various Alkylamide Groups at Chain Ends

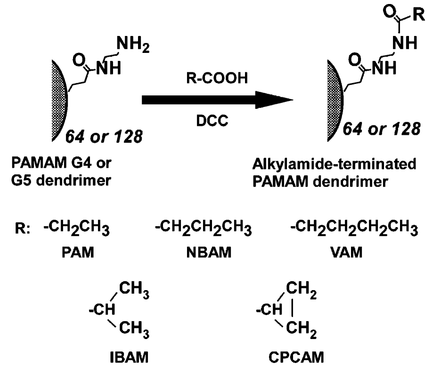

Table 1. Characterization of Various Alkylamide-Terminated Dendrimers[a]

dendrimer	no. of chain ends	no. of terminal alkylamide groups incorporated
PAM-G5	128	130.5
NBAM-G5	128	131.5
VAM-G5	128	130.3
NBAM-G4	64	64.5
IBAM-G4	64	64.2
CPCAM-G4	64	63.8

[a] Determined by ^{1}H NMR.

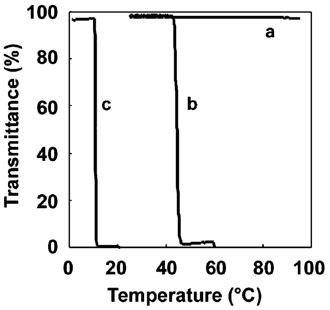

Figure 1. Effect of temperature on transmittance of PAM-G5 (a), NBAM-G5 (b), and VAM-G5 (c) dissolved in 10 mM phosphate solution (10 mg/mL, pH 9.0).

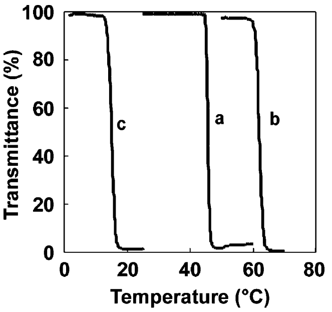

Figure 2. Effect of temperature on transmittance of (a) IBAM-G4, (b) NBAM-G4, and (c) CPCAM-G4 dissolved in 10 mM phosphate solution (10 mg/mL, pH 9.0).

dendrimer, as 0.84, 0.59, and 0.24, respectively, using Broto's fragmentation method.[13] Therefore, the hydrophobicity of peripheral alkylamide groups is inferred to increase in the order of CPCAM-G4 < IBAM-G4 < NBAM-G4. In fact, Ito reported that LCSTs of poly(*N*-cyclopropylacrylamide), poly(*N*-isopropylacrylamide), and poly(*N*-*n*-propylacrylamide) decreased with the same order, suggesting that hydrophobicity of the alkylamide groups can determine LCST for these polymers.[10]

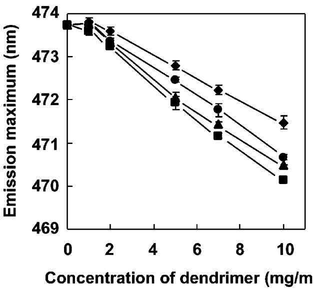

Figure 3. Emission maxima for PyCHO in aqueous solutions of hydroxyl-terminated PAMAM-G4 (diamonds), IBAM-G4 (circles), NBAM-G4 (triangles), and CPCAM-G4 (squares) at 4 °C.

However, in the case of the alkylamide-terminated dendrimers, their LCST is not simply determined by hydrophobicity of the terminal alkylamide groups because CPCAM-G4 dendrimer showed the lowest cloud point temperature among these modified G4 dendrimers. Considering that conformational freedom of CPCAM groups is much lower than that of IBAM or NBAM groups, CPCAM groups might be highly concentrated in the peripheral region of the dendrimer.

We examined NMR relaxation times (T_1) for the terminal alkylamide groups attached to the dendrimers to obtain information on their mobility. The T_1 values for the terminal methyl/methylene protons of NBAM, IBAM, and CPCAM groups in the periphery of the dendrimers were evaluated to be 1.366, 0.882, and 0.682 s, respectively, whereas those for the same protons of the corresponding carboxylic acids used for the preparation of these alkylamide-terminated dendrimers were 2.973, 2.331, and 2.569 s, respectively. This result indicates that the CPCAM groups are most tightly packed in the dendrimer periphery and have the lowest mobility among these terminal groups. Therefore, it is likely that the dendrimer periphery crowded with CPCAM groups can enable their efficient interaction, resulting in the phase separation at the lowest temperature.

We further examined hydrophobicity of these alkylamide-terminated PAMAM G4 dendrimers using 1-pyrenecarboxaldehyde (PyCHO) to obtain insight into the low phase separation temperature of the CPCAM-G4 dendrimer. This molecule is known to change emission maxima (λ_{max}) depending on polarity of the solvent.[14] As shown in Figure 3, PyCHO displayed a more marked blue shift of λ_{max} in the presence of alkylamide-terminated dendrimers than in the case of hydroxyl-terminated G4 dendrimer. These dendrimers possess the same interior. Therefore, the large blue shift of the alkylamide-terminated dendrimers might indicate higher hydrophobicity of the peripheral region of these dendrimers. In addition, the CPCAM-G4 dendrimer exhibits a slightly larger blue shift, which is indicative of the slightly higher hydrophobic nature of this dendrimer's surface. As already mentioned, the log *P* values for these alkylamide groups indicate that CPCAM group has the lowest hydrophobicity among these alkylamide groups. However, CPCAM groups are most densely packed in the dendrimer periphery among them. Probably, the dense packing of the CPCAM groups in the dendrimer periphery might reduce contact with water molecules efficiently and form the hydrophobic surface.

From a practical viewpoint, control of LCST of thermosensitive dendrimers is very important. For thermosensitive polymers with a linear structure, copolymerization with monomers having appropriate hydrophilicity or hydrophobicity is widely used to control LCST.[9,12,15] Therefore, finally, we examined controllability of LCST of the dendrimer by adjusting the ratio of peripheral alkylamide groups. Three kinds of PAMAM G4 dendrimer having IBAM and CPCAM groups of 15 and 49, 29

Table 2. Preparation of IBAM-CPCAM-G4 Dendrimers[a]

	in feed (mol %)		obtained (mol %)	
dendrimer	isobutyric acid	cyclopropanecarboxylic acid	IBAM	CPCAM
IBAM-CPCAM(1/3)-G4	25	75	23.5	76.5
IBAM-CPCAM(1/1)-G4	50	50	45.9	54.1
IBAM-CPCAM(3/1)-G4	75	25	70.3	29.7

[a] Determined by ^{1}H NMR.

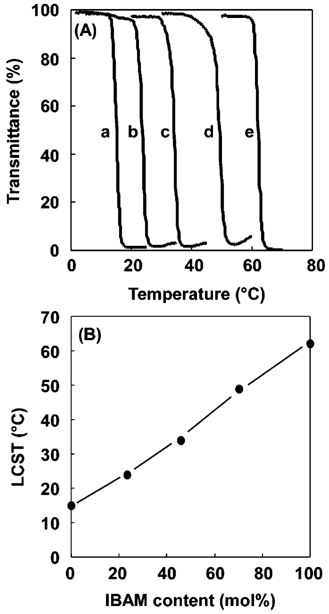

Figure 4. Influence of the IBAM/CPCAM ratio in the periphery on cloud point of IBAM-CPCAM-G4 dendrimers. (A) Effect of temperature on transmittance of dendrimers with IBAM/CPCAM (mol/mol) ratios of (a) 0/1, (b) 1/3, (c) 1/1, (d) 3/1, and (e) 1/0. The dendrimers were dissolved in 10 mM phosphate solution (10 mg/mL, pH 9.0). (B) Cloud point of IBAM-CPCAM-G4 dendrimers as a function of the content of IBAM groups (mol %) in the peripheral alkylamide groups.

and 35, and 45 and 19 per dendrimer were prepared with the PAMAM G4 dendrimer by adjusting the ratio of the corresponding carboxylic acids during their condensation (Table 2). Figure 4A depicts the temperature dependence of transmittance of the dendrimers that have IBAM and CPCAM groups at varying ratios. These dendrimers exhibited change in transmittance at different temperatures, indicating that their cloud point is dependent on the ratio of IBAM/CPCAM in the periphery. Despite the large difference of temperature regions of their cloud points, these dendrimers showed a sharp change in water solubility. Figure 4B represents the cloud points of the dendrimers with IBAM and CPCAM groups as a function of the percentage of IBAM groups in the terminal alkylamide groups. Apparently, the cloud point of the dendrimer increased with increasing IBAM fraction. This result indicates that dendrimers that exhibit a response at a desired temperature are obtainable by controlling the relative composition of terminal alkylamide groups.

As shown in this study, surface modification with alkylamide groups that are common structural units with well-known thermosensitive polymers with a linear structure provides temperature-sensitive properties to PAMAM dendrimers. Previously, we demonstrated that modification with IBAM groups, which is a structural unit of thermosensitive polymer poly(*N*-vinylisobutyramide), can provide temperature-sensitive properties to PAMAM dendrimers.[8] Through this study, we further generalized this strategy for production of thermosensitive dendrimers.

Dendrimers that exhibit LCST are thermosensitive polymers with highly unique features, such as having a three-dimensional structure and being monodispersed. In addition, dendrimers that exhibit thermosensitive properties in aqueous solutions are of importance from the practical standpoint. For example, dendrimers with LCST around physiological temperature could be used for site-specific drug delivery because such dendrimers could release drug and/or control interaction with cells at the target site heated. Only few dendrimers have been reported to exhibit temperature-sensitive properties so far.[8,16,17] Therefore, findings obtained in this study provide an efficient method to produce thermosensitive dendrimers and lead to a generation of a new family of thermosensitive polymers.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org

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