J|A|C|S
COMMUNICATIONS

Published on Web 05/16/2007

Temperature Sensitivity Control of Alkylamide-Terminated Poly(amidoamine) Dendrimers Induced by Guest Molecule Binding

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A particularly interesting aspect of dendrimers is their use as nanocapsules.[1] Originating from their highly branched backbone structure, dendrimers take on a ball-like shape, which gives them an interior which can accommodate small molecules, such as anticancer drugs and dyes, and metal nanoparticles.[1,2]

The periphery of dendrimers is a key moiety to improve the encapsulation ability of the dendrimers and/or provide additional functionality to dendrimers. The capability of dendrimers to retain guest molecules can be improved by construction of a shell consisting of bulky amino acid residues[2b] or cross-linking of chain terminals in the dendrimer periphery.[2e] In addition, stimuli sensitivity, which is an important property of dendrimers as functional nanocapsules, has been imparted by introduction of stimuli-sensitive groups to the periphery of dendrimers.[3]

Recently, we developed an efficient strategy for sensitization of dendrimers to temperature: surface modification of dendrimers with alkylamide groups that are common with thermosensitive polymers.[4] For example, we introduced an isobutyramide (IBAM) group, a common structural unit with thermosensitive poly(*N*-vinylisobutyramide),[5] to every chain end of poly(amidoamine) (PAMAM) and poly(propylenimine) dendrimers. These IBAM-terminated dendrimers exhibited lower critical solution temperature (LCST) at a specific temperature depending on their generation.[4a] Furthermore, interaction of peripheral alkylamide groups was demonstrated to be mainly responsible for their thermosensitive properties.[4b,c]

Alkylamide-terminated dendrimers can be regarded as an entirely new polymer type, with a thermosensitive surface and an interior that are available for guest molecule encapsulation. With the goal of development of functional molecules that can serve as nanocapsules with temperature-controlled properties, we investigated in this study how binding of guest molecules influences the temperature-sensitive properties of alkylamide-terminated PAMAM dendrimers.

We obtained IBAM-terminated and propionamide (PAM)-terminated PAMAM G5 dendrimers, which are designated as IBAM-G5 and PAM-G5, respectively (Figure 1), as previously reported.[4a,c] In addition, the PAMAM G5 dendrimer having an acetamide (Ac) group at every chain end, which is designated as Ac-G5, was synthesized using the reaction of the amine-terminated PAMAM G5 dendrimer with excess acetic anhydride, as reported previously.[6] Analyses using ^{1}H and ^{13}C NMR indicated that essentially every chain end of the dendrimers was combined with the corresponding alkylamide group (see Supporting Information).

First, we investigated the influence of binding of guest molecules to the dendrimers on their thermosensitivity. Rose bengal (RB) was chosen as the guest because this dye molecule exhibits high affinity to the interior of PAMAM dendrimers and has been widely used for this purpose.[2b,e,3b] As shown in Figure 2A, the IBAM-G5 dendrimer exhibited a cloud point, which is indicative of LCST, around 36 °C at pH 7.4 in the absence of RB. However, when this

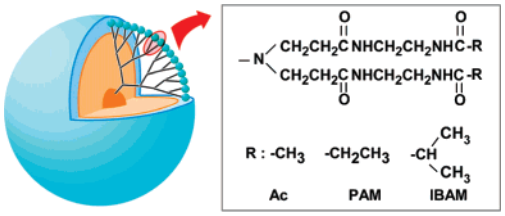

Figure 1. Structures of alkylamide-terminated PAMAM G5 dendrimers, which have 128 terminal groups.

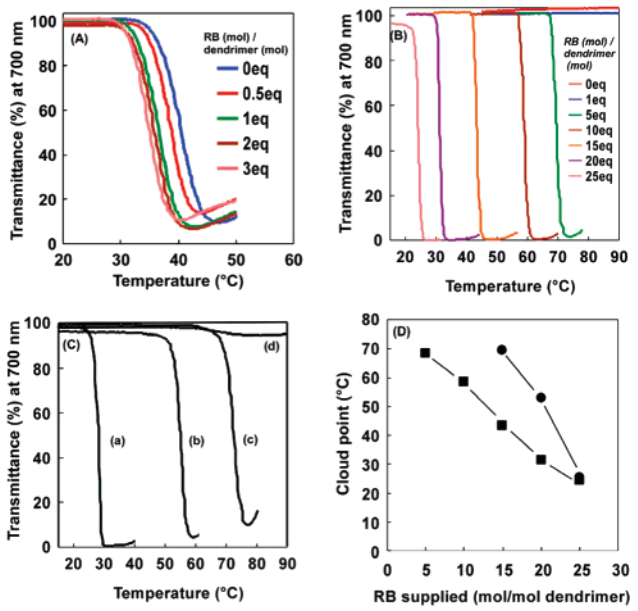

Figure 2. Effect of RB binding on temperature sensitivity of various alkylamide-terminated dendrimers in phosphate solution (50 mM). Temperature dependence of light transmittance for solutions of the IBAM-G5 dendrimer (53 μM) with varying equivalents of RB at pH 7.4 (A) and 6.0 (B). (C) Temperature dependence for solutions (pH 6.0) of PAM-G5 (a−c) and Ac-G5 (d) dendrimers (53 μM) with varying equivalents of RB: 25 (a), 20 (b) 15 (c), and 25 (d) equiv per dendrimer. (D) Cloud points of IBAM-G5 (squares) and PAM-G5 (circles) dendrimers encapsulating RB as a function of the amount of RB supplied to the dendrimer.

dye molecule was present, the dendrimer's cloud point differed. Apparently, the cloud point decreased with increasing amount of RB per dendrimer. Addition of 1 equiv of RB per dendrimer markedly lowered the cloud point by about 4 °C. Probably, the RB molecule, which possesses a hydrophobic backbone with hydroxyl and carboxyl groups, binds to the dendrimer interior through electrostatic interaction and/or hydrogen bonding[2c,7] and increases its hydrophobicity. However, further addition of RB became less effective on the cloud point. Electrostatic force is known to contribute to the strong interaction between the dendrimer backbone and RB.[2e,3b] However, because tertiary amines of the

10.1021/ja0711718 CCC: $37.00

dendrimer backbone become protonated below pH 7.0,[8] the affinity of RB to the dendrimer interior without protonation is not very strong at pH 7.4.

Results of a previous study demonstrated that the affinity of RB to the PAMAM dendrimer interior increased considerably at mildly acidic pH.[2e,3b] For example, PAMAM dendrimers with terminal poly(ethylene glycol) chains was shown to have 9 and 27 sites for RB binding at pH 7.4 and 6.0, respectively.[2e] Therefore, we further investigated the binding of RB on thermosensitivity of the IBAM-terminated dendrimer under a weakly acidic condition.

As depicted in Figure 2B, the IBAM-G5 dendrimer dissolved in aqueous solution at pH 6.0 over the experimental temperature range when RB was not present in the solution. It dissolved because of the protonation of tertiary amines of the dendrimer interior, which promoted hydration of the dendrimer molecule.[4a] However, when RB is present, the dendrimer exhibits a cloud point. Although 1 equiv of RB per dendrimer was insufficient for the appearance of a cloud point, the presence of more than 5 equiv of RB per dendrimer rendered the dendrimer temperature-sensitive. In addition, the cloud point of the dendrimer decreased considerably, concomitant with the increasing ratio of RB to the dendrimer. An increase of supplied RB to the dendrimer from 5 to 25 equiv decreased the cloud point by about 50 °C. This result indicates that, although the guest molecules bound to the dendrimer interior strongly affect the temperature sensitivity of the dendrimer, the dendrimer retains temperature sensitivity.

We further examined the effect of RB binding on thermosensitive properties of PAMAM dendrimers with different alkylamide groups in the periphery (Figure 2C). A sharp decrease in the transmittance at a specific temperature was apparent for the solution of the PAM-G5 dendrimer with RB. Its cloud point was dependent on the amount of RB supplied to the dendrimer. However, the Ac-G4 dendrimer solution did not exhibit a marked change in light transmittance, even in the presence of 25 equiv of RB per dendrimer.

The effect of RB binding on the dendrimer cloud point was compared between PAM-G5 and IBAM-G5 dendrimers (Figure 2D). Their cloud points decreased with increasing RB added to the dendrimers. However, the IBAM-G5 dendrimer exhibited a cloud point lower than that of the PAM-G5 dendrimer when the same amount of RB was supplied. These dendrimers possess the interior of the same structure. Therefore, it is apparent that hydrophobic−hydrophilic balances of not only the peripheral alkylamide groups of the dendrimer but also the whole dendrimer−RB complex controlled its temperature sensitivity.

Finally, we examined the possible use of thermosensitive dendrimers for separation of the guest molecules. The IBAM-G5 dendrimers were mixed with 25 equiv of RB in 50 mM phosphate solution at pH 6.0 and incubated below or above the cloud point. Then, the solution was centrifuged and the absorbance of the supernatant was measured (Figure 3). The spectra were fundamentally identical before and after centrifugation when the incubation was carried out below the cloud point. However, the centrifugation was done after incubation above the cloud point, and the supernatant showed a very low level of absorption derived from RB. As judged from absorbance at 556 nm, about 97% of RB molecules were recovered with the dendrimer using this procedure, indicating that the dendrimers precipitated with retaining RB molecules. Recently, temperature-sensitive micelles of biaryl-based amphiphilic dendrons were used for extraction of pyrene from solutions.[9] However, its complete recovery was not obtained, probably because of the dynamic property of the micelles. Therefore, the excellent separation achieved by the IBAM-terminated dendrimer is noteworthy. In addition, the fact that the cloud point of the dendrimer−guest complexes depends on pH (Figure 2A,B) indicates the possibility of the same separation by changing pH of the solution (see Supporting Information).

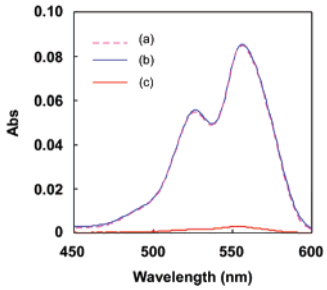

Figure 3. Absorption spectra for IBAM-G5 with 25 equiv of RB dissolving in 50 mM phosphate-buffered solution (pH6.0) after incubation at 15 °C without (a) and with (b) subsequent centrifugation, and (c) after incubation at 70 °C and subsequent centrifugation. The solution of IBAM-G5 (53 μM) with RB (1.33 mM) was incubated at a given temperature for 40 min and then centrifuged. The supernatant was diluted 665 times with the same buffer, and the absorption spectrum of the diluted solution was measured.

An important advantage for the alkylamide-terminated dendrimers should be possession of the thermosensitive periphery and inner space capable of encapsulating guest molecules. These dendrimers can encapsulate guest molecules and retain thermosensitive properties. The amount of RB molecules held in the dendrimer interior affected their temperature sensitivity, which means that the association with guest molecules is useful to control thermosensitivity of the dendrimers, leading to a novel strategy for temperature sensitization of dendrimers. We observed that the PAM-G5 dendrimer, which does not exhibit LCST at any pH and is fundamentally temperature-insensitive,[4c] became temperature-sensitive after complexation with RB. Findings obtained through this study might contribute to the generation of new types of thermosensitive polymers and consequent expansion of the range of dendrimer applications.

Supporting Information Available: Experimental procedures, characterization data, and pH-induced separation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0711718