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# Preparations and Characterizations of Nano-Sized Complexes Consisting of Stimuli-Responsive Block Copolymers and PAMAM Dendrimers

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We have studied the preparations and characterizations of nano-sized complexes derived from stimuli-responsive AB- or ABA-type block copolymers and G3 poly(amidoamine) dendrimers. The block copolymers consisted of thermo-responsive poly(*N*-isopropylacrylamide) and poly(methacrylic acid), respectively. The nano-sized complexes were consequent on the electrostatic interactions between carboxyl groups of the block copolymers and amino groups of dendrimers in aqueous solutions. We confirmed the effective nano-sized complex formation by dynamic light scattering measurement and transmission electron microscope. Interestingly, the nano-sized complex derived from ABA-type triblock copolymer had smaller size than that from AB-type diblock copolymer. The condensation reactions between the block copolymers and the dendrimers effectively improved the stability of the nano-sized complexes in aqueous solutions. Furthermore, we also confirmed the sensitive response in temperature change.

Key words: Atom Transfer Radical Polymerization, Stimuli-Responsive Block Copolymers, PAMAM Dendrimer, Complex Formations

## INTRODUCTION

The block copolymers that their segments show different characters can form very interesting associates or micelles by self-assembly in the optimal conditions.[1-3] The characteristics of them are tunable by the kinds of components, chemical structures, and chain lengths of each blocks.[4,5]

Recently, stimuli-responsive polymers have attracted much attention as the components for micelle- or associates-forming block copolymers. Because the resulting stimuli-responsive polymeric micelles become candidates for fabrication of the novel drug delivery systems with higher potentials. Actually, temperature-responsive polymeric micelles can be easily derived from poly(*N*-isopropylacrylamide) (PNIPAAm) -based block copolymers with hydrophobic segment.[6] They can form micellar structure below Lower Critical Solution Temperature (LCST). Under heated condition, the thermo-responsive shell layers surrounding the hydrophobic cores effectively dehydrate and the micelles would turn into hydrophobic aggregates. Using this unique system, we could selectively accumulate the drug carrier by local heating. Not only the surface character and association behavior but also the drug loading or release would be controlled.[7,8]

Moreover, polyion complex formation is also practical as the driving force for another unique polymeric micelles or associates. The polyion complex micelles are formed by the electrostatic interactions between oppositely charged block copolymers in aqueous solutions.[9] The ionic block copolymers with a polyethylene oxide (PEO) segments exhibit exclusively self-assemble behavior. Moreover, their micelles are very stable nanometer-sized spheres with mono-disperse. In other cases, the associates by charged block copolymers could be formed with the multivalent metal ions or supermolecules.[10-15]

In this study, we prepared the nano-sized complexes derived from ionic stimuli-responsive block copolymers with carboxyl groups and poly(amidoamine) (PAMAM) dendrimers with amino groups by the electrostatic interactions and characterized them in the aqueous media. Figure 1 shows schematic representation of aggregation behaviors of stimuli-responsive block copolymers and dendrimer in aqueous solution. The stimuli-responsive block copolymers consisted of two segments, PNIPAAm and poly(methacrylic acid) (PMAA) formed the AB- or ABA-type block copolymers. Furthermore, the effective nano-sized complex stabilization was carried out by the condensation reactions between the block copolymers

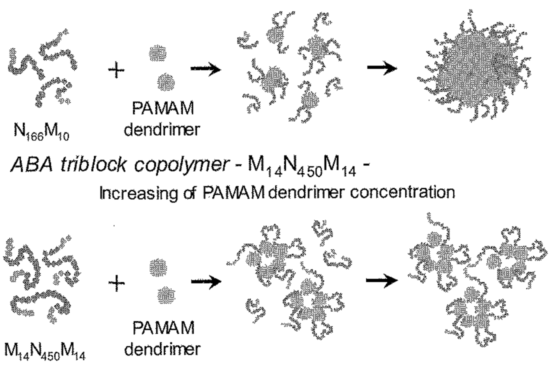

Figure 1. Schematic representation of the aggregation behaviors of stimuli-responsive block copolymers and PAMAM dendrimer.

and dendrimers.

Dendrimers are known to act as host for guest molecules such as metal ions, organic and inorganic compounds.[16,17] Recently, it was reported that the complex derived from PAMAM dendrimers and block copolymers with a PEO segment showed high stability in aqueous solutions by surface modification of PAMAM dendrimer.[13-15] It is expected that aggregation behavior of dendrimer would be controlled by using the stimuli-responsive copolymers as the modifier.

## EXPERIMENTAL SECTION

**Materials.** PNIPAAm was kindly supplied from Kojin (Tokyo, Japan) and purified by recrystallization from *n*-hexane. *t*-Butylmethacylate (*t*BMA) was purchased from Wako Pure Chemicals (Osaka, Japan) and purified by distillation under reduced pressure. 1-Ethyl-3-(3-dimethylamino propyl)carbodiimide hydrochloride (EDC) was obtained from Tokyo Chemical Ind. (Tokyo, Japan) and used without purification. Other chemicals for polymer synthesis and characterization were of analytical grade and used without further purification.

PNIPAAm-*b*-PMAA as AB-type diblock copolymer and PMAA-*b*-PNIPAAm-*b*-PMAA as ABA-type triblock copolymer were prepared by hydrolysis of corresponding precursors, PNIPAAm-*b*-P*t*BMA and P*t*BMA-*b*-PNIPAAm-*b*-P*t*BMA. They were synthesized by atom transfer radical polymerization (ATRP) according to the literature.[18] PNIPAAm-*b*-PMAA contains 166 units for a PNIPAAm segment and 10 units for a PMAA one, abbreviated to as $N_{166}M_{10}$. The molecular weight and its distribution are $M_n$ = 18900 and $M_w/M_n$ = 1.32, respectively. PMAA-*b*-PNIPAAm-*b*-PMAA is 450 units for a PNIPAAm segment in a middle block and 14 units for each PMAA segments in end blocks, referred to as $M_{14}N_{450}M_{14}$. The molecular weight and its distribution are $M_n$ = 18900 and $M_w/M_n$ = 1.32, respectively. G3 PAMAM dendrimer was purchased from Sigma-Aldrich (St. Louis, USA). The solvent was evaporated under reduced pressure before use and diluted with 50 mM tris HCl buffer solutions at a concentration of 10 mg/mL with adjusted pH. The theoretical molecular weight of the dendrimer is $M_w$ = 6906, and it has 32 primary groups $-NH_2$ and 30 tertiary groups -N=.

**Preparations of Nano-sized Complexes.** Nano-sized complexes were prepared in the following way. First, the block copolymers were dissolved in 50 mM tris HCl buffer solutions at a concentration of 1.0 mg/mL with adjusted pH. Then, the dendrimer solution described above was added to the block copolymer solutions with stirring. As described below, we defined the molar mixing ratio as $[-NH_2]/[-COOH]$.

**Stabilization of Nano-sized Complexes.** For stabilization of nano-sized complex, adequate amount of the condensation agent, EDC was added to the nano-sized complexes solution and it was stirred for 12 h at 4 °C. After the reaction, nano-sized complexes were purified by repeated washing with purified water.

**Dynamic light scattering spectroscopy(DLS).** The DLS measurements were conducted with FPAR-1000HL spectrometer (Otsuka Electronics Co., Ltd., Osaka, Japan) to estimate the complex sizes and associates formation.

**Electrophoretic light scattering spectroscopy.** The z-potentials of the nano-sized complexes were estimated using ELS-6100 (Otsuka Electronics Co., Ltd., Osaka, Japan).

**Transmission electron microscopic (TEM) studies.** The TEM were also performed for evaluation of association size on a JEOL JEM-3010 (JEOL Ltd., Tokyo, Japan) at acceleration voltage of 300 kV. The sample was prepared by air-drying of the diluted solution on the collodion films.

**Investigation of thermo-responsive behavior by turbidity measurement.** The thermo-responsive profiles were investigated by the transmittance measurements using a JASCO V-55 UV-Vis spectrometer (JASCO Corporation, Tokyo, Japan).

## RESULTS AND DISCUSSION

**Nano-sized complex formations derived from AB-type block copolymers and dendrimers.** First, DLS measurements were carried out to investigate the nano-sized complex formations by simple mixing of the block copolymers and the dendrimer solutions. Figure 2 shows the dendrimer concentration dependence of the diameters of the nano-sized complexes derived from $N_{166}M_{10}$ and dendrimer. As seen in Fig. 2, the diameters steeply increased with the $[-NH_2]/[-COOH]$ increasing, and then were equilibrated at the specific $[-NH_2]/[-COOH]$ in every sample. The equilibrated values of the diameters closely depended on pH values of aqueous solutions used for the complex formation.[19] The profiles of scattering intensities were also same to results obtained the dendrimer concentration dependence of diameters in Fig. 2.

Both $-NH_2$ and -N= become $-NH_3^+$ and $-NH^+=$ at pH = 6 and interact with all $-COO^-$ of $N_{166}M_{10}$ (Figure 3). As described above, the number of $-NH_3^+$ and $-NH^+=$ are 32 and 30 in a dendrimer molecule, respectively. As seen in Fig. 2, the diameter reached the equilibrated value when $[-NH_2]/[-COOH]$ was around 0.5. This result means the not only $-NH_3^+$ but also almost same number of $-NH^+=$ would participate in the complex formation. That is why the threshold was observed to be near 0.5 in the solution adjusted pH = 6.

Taking the stoichiometry of the ionic interaction into

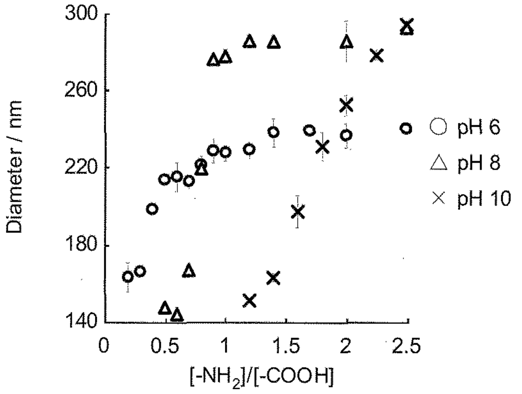

Figure 2. Diameters (a) of nano-sized complexes for $N_{166}M_{10}$ solutions as a function of the PAMAM dendrimer concentration at pH 6 (circles), 8 (triangles), 10 (crosses) ; polymer concentration = 0.1 w/v%, $T$ = 21 °C.

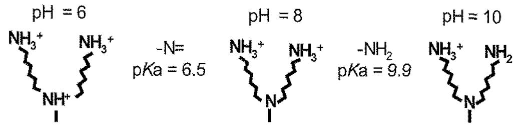

Figure 3. Schematic representation of protonations of PAMAM dendrimer dependent on pH.

the consideration, [-NH$_2$]/[-COOH] of around 1 at pH = 8 and 2 at pH = 10 could be easily understood. The p*K*a values of -NH$_2$ and -N= in dendrimer are 6.5 and 9.9 respectively (Fig. 3). At pH = 8, -NH$_3^+$ could only interact with -COO$^-$. At pH = 10, a half –NH$_3^+$ could react because the pH value is very near p*K*a of -NH$_2$. The results clearly mean that nano-sized complexes formed through electrostatic interaction.[20]

-COO$^-$ of N$_{166}$M$_{10}$ are 10 in each chain above pH = 6. There are 32 -NH$_2$ and 30 -N= in dendrimer that protonate or deprotonate dependent on pH. -COO$^-$ of N$_{166}$M$_{10}$ interacts with protonated amino groups of dendrimer by electrostatic interactions. And then, the remaining deprotonated amino groups would induce the association between the complexes gradually by hydrogen bonds and reach thermodynamically equilibrated complexes resultantly. Fig. 2 indicated that the diameters of nano-sized complexes obtained at pH = 8 and 10 were larger than that at pH = 6. This results suggests that the remained -N= were more than that at pH = 6. The -N= could contribute hydrogen bonding to form larger associates described above.

**Nano-sized complex formations derived from ABA-type block copolymers and dendrimers.** Figure 4 shows the scattering intensity and diameters of the nano-sized complexes derived from ABA-type block copolymer M$_{14}$N$_{450}$M$_{14}$ in dependence on [-NH$_2$]/[-COOH]. The profiles were totally different from those from N$_{166}$M$_{10}$. In terms of the stoichiometry between carboxyl and amino groups, [-NH$_2$]/[-COOH] values that reached equilibrated values were larger than those from N$_{166}$M$_{10}$. As indicated in the same figure, the intensities as well as diameters were considerably smaller than those from N$_{166}$M$_{10}$. These results suggest that the constituting number of M$_{14}$N$_{450}$M$_{14}$ and dendrimers would be fewer in resulting complexes. Interestingly, the complex diameters were almost same as around 100 nm independent of [-NH$_2$]/[-COOH] ratio.

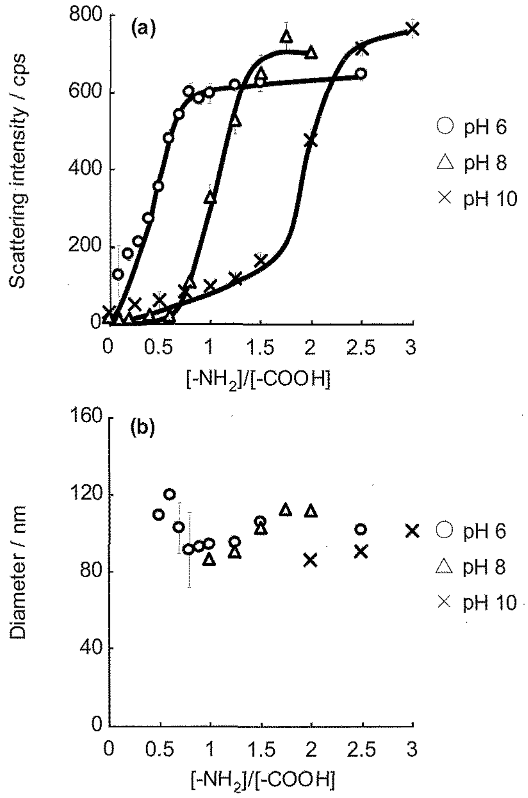

Figure 4. Scattering intensities (a) and diameters (b) of nano-sized complexes for M$_{14}$N$_{450}$M$_{14}$ solutions as a function of the PAMAM dendrimer concentration at pH 6 (circles), 8 (triangles), 10 (crosses) ; polymer concentration = 0.1 w/v%, $T$ = 21 °C.

Moreover, the z-potentials at the [-NH$_2$]/[-COOH] = 1.0 and 1.5 were -3.0 mV and -1.9 mV (pH = 8). These results suggest the presence of free -COO$^-$. The one side segment of the block copolymer would only react with the dendrimer and some free mobile chains with -COO$^-$ would remain. It would be effectively interfered by the sterically hindrance and repulsion of negative charge. That is why the scattering intensity and diameters were considerably low and small compared with those from N$_{166}$M$_{10}$.

**Stabilizations of nano-sized complexes.** Prior to the stabilization, we have checked the stability by the turbidity observation. In the condition with pH = 8, the sample solution from N$_{166}$M$_{10}$ was slightly opaque due to the larger complex size. In the same condition, the solution prepared with M$_{14}$N$_{450}$M$_{14}$ looks very slightly blue based on the light diffraction. At pH = 13, both samples solution prepared with N$_{166}$M$_{10}$ and M$_{14}$N$_{450}$M$_{14}$ were completely transparent. Since this condition is over p*K*a value of -NH$_2$ in the dendrimer, the complexes dissociate into the block copolymer with -COO$^-$ and the non-charged dendrimers. Then, to improve the stability in wide pH condition, we carried out the chemical reaction.

After the reaction, as expected, the both solutions have maintained similar properties and the nano-sized complexes could not dissociate even though it was exposed at pH = 13. Obviously, the amide bonding

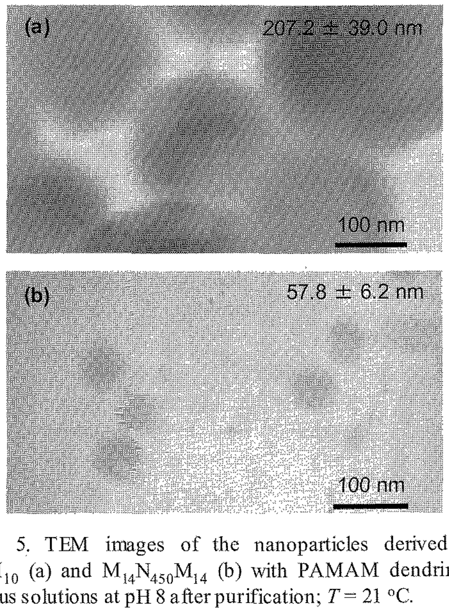

Figure 5. TEM images of the nanoparticles derived from N$_{166}$M$_{10}$ (a) and M$_{14}$N$_{450}$M$_{14}$ (b) with PAMAM dendrimer in aqueous solutions at pH 8 after purification; $T$ = 21 °C.

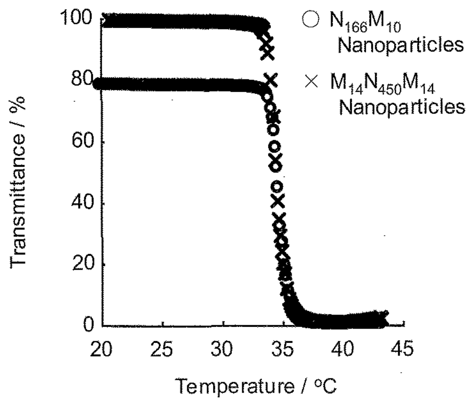

Figure 6. Transmittance measurements of nanoparticles derived from $N_{166}M_{10}$ (circles) and $M_{14}N_{450}M_{14}$ with PAMAM dendrimer (crosses) at pH 8 ; programming rate = 1 °C/min.

formation contributed to improve the stability in severe condition.

We also studied the TEM of the nano-sized complexes and the obtained images were shown in Figure 5. They supported the round-shaped complexes and the reasonable sizes of the complexes. The sizes obtained from TEM were smaller than those from DLS because of drying of samples.

**Thermo-responsive profiles of the nano-sized complexes.** We examined the thermo-responsive profiles of prepared nano-sized complexes in aqueous solutions by turbidity measurements and the results are shown in Figure 6. The nano-sized complexes stabilization shows the sensitive responsive behavior. The cloud points are the almost same as that of the starting $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ themselves. In some case, clout points of the thermo-responsive polymer were changeable by the introduction of hydrophilic or hydrophobic moieties.[21] For example, the introduction of hydrophobic moieties tends to shift to lower temperature. In this study, we observed no change in the clout points and it might be due to the relatively hydrophilic nature of the amide bonding and dendrimer surface.

## CONCLUSIONS

We succeeded in preparation of the nano-sized complexes comprised of the stimuli-responsive $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ and dendrimers by the electrostatic interaction. Interestingly, the sizes of the obtained complexes were different depending on the types of starting block copolymers. The nano-sized complexes derived from $M_{14}N_{450}M_{14}$ had smaller size than that from $N_{166}M_{10}$. It might be due to the difference in the complex formation process. Furthermore, we could improve the stability by simple reaction. The stabilized nano-sized complexes were not disrupted in higher pH. Moreover, these obtained nano-sized complexes exhibited sensitive thermo-responsive properties.

The polymer modification with stimuli-response in this study can surely contribute to the bio-conjugate chemistry and drug delivery system because of not only the easy preparation but also the size and charge control of the complexes. In near future, we will report the complex formation process in detail.

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