A Novel Approach to Preparing Multi Stimuli-Responsive Nano-Assembly by A Simple Mixing of Block Copolymers

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Introduction: The ever-growing needs of mankind have led to discovery of new materials with tailor-made properties. 'Smart' polymers, being easily synthesizable materials whose properties can be controlled to a large extent, are thus indispensable to us. Nano-assembly consisting of these smart polymers has been applied for drug delivery system as biomaterials. One of the problems in protocols for preparing smart nano-assembly is, however, that organic solvents have been required for the formation of the assembly. This process also takes time, resulting in leaking of encapsulated drug from the assembly. Besides, adding second moiety (*e.g.*, pH-sensitive etc.) into the assembly is not easy once polymerization has done. We have developed a simple method that facilitate a preparation of double thermo-responsive block copolymers, composed of poly(*N*-isopropylacrylamide) (PNIPAAm) PNIPAAm block and it's copolymer block (Kotsuchibashi Y. J Polym Sci Part A: Polym Chem. 2008;46:6142-6150. Kotsuchibashi Y. J Colloid Interface Sci. 2009;336:67-72.). Upon heating, nano-assembly successfully formed in an aqueous media. In addition, additional heating induced aggregation of the assembly. In this study, we propose here a novel approach to integrating negative charged moiety into the double thermo-responsive assembly with a simple mixing of the two block copolymers solution(Fig. 1).

Methods: A series of block copolymers was synthesized by atom transfer radical polymerization(ATRP) using 2-ethylbromoisobutylate, Copper(I) bromide and tris(2-dimethylaminoethyl)amine(Me_6TREN). NIPAAm, *N*-(hydroxymethyl)acrylamide (HMAAm), and 2-acrylamido-2-methyl-1-propanesulphonate (AMPS) were used as monomers. The obtained block copolymer solutions were mixed and the solution temperature was raised for forming multiple stimuli-responsive assembly. These properties were estimated using ^{1}H-NMR spectra with 300 MHz(JEOL, Tokyo, Japan), UV-vis spectrometer (Jasco V-550, Tokyo, Japan) and dynamic light scattering (Otsuka Electronics Photal FPAR-1000HL, Osaka, Japan).

Results: The obtained block copolymers were estimated by ^{1}H-NMR ($PNIPAAm_{248}$-*b*- P($NIPAAm_{419}$-*co*-$HMAAm_{113}$) and $PNIPAAm_{248}$-*b*-P($NIPAAm_{461}$-*co*-$AMPS_{108}$) were abbreviated as $N_{248}bN_{419}H_{113}$ and $N_{248}bN_{461}A_{108}$ respectively, where right number are the monomer unit in block copolymers). The copolymerized monomer content (HMAAm or AMPS) were similar value with in feed. $N_{248}bN_{419}H_{113}$ showed double thermo-responsive behavior as shown in Fig.2(A). On the other hand, $N_{248}bN_{461}A_{108}$ showed only one step

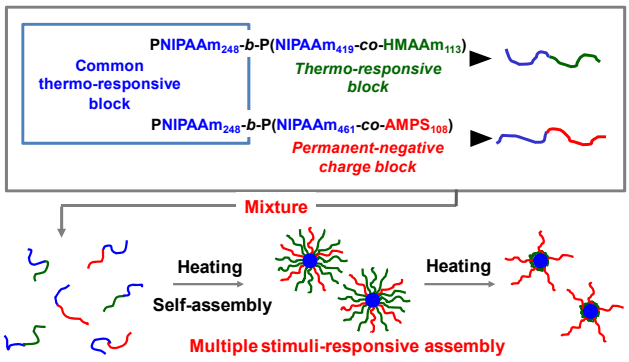

Figure 1. A preparation protocol of multi stimuli-responsive assembly by a simple mixing and heating without organic solvents.

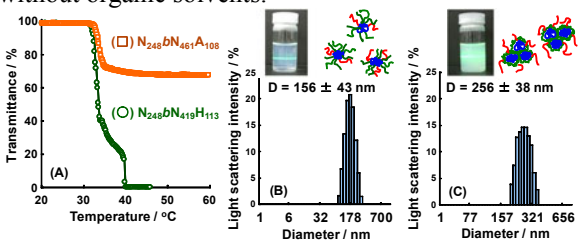

Figure 2. (A) Temperature dependence of transmittance for 0.5 w/v% of block copolymers in 100 mM $NaCl_{aq.}$, (B) diameter for 0.1 w/v% of mixted block copolymers (N*b*NH: N*b*NA = 95:5 v/v%) in 100 mM $NaCl_{aq}$ at 40 °C, (C) 45 °C.

thermo-responsive aggregation due to the hydrophilic AMPS prevented the further aggregation. In addition, a simple mixing and heating of two solutions of different block copolymers successfully formed the multi stimuli-responsive assembly. Thus, the proposed protocol for preparing stimuli-responsive nano-assembly enable to form nano-assembly in an aqueous media and induce their aggregation upon second stimulus(Fig. 2 (B) and (C)).

Conclusions: We synthesized multi stimuli-responsive block copolymers which had a common thermo-responsive block for forming a core of the nano-assembly only by a simple mixing and heating. Since they also have stimuli-responsive polymers in the shell, second heating induced the aggregation of the assembly. We also succeeded in employing negative charge groups into the assembly only by co-mixing the block copolymer with AMPS. Since the proposed protocol enable to customize the function of the assembly by a simple mixing and heating of the selected block copolymers, it would promise a wide range of application such as carriers for drug delivery.