Introducing Reactive Carboxyl Side Chains Retains Phase Transition Temperature Sensitivity in *N*-Isopropylacrylamide Copolymer Gels

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ABSTRACT: Introduction of significant quantities of functional carboxyl groups into temperature-responsive poly(*N*-isopropylacrylamide) (PIPAAm) hydrogels without compromising their intrinsic temperature sensitivity has proven difficult. We have overcome this problem by incorporating the newly synthesized 2-carboxyisopropylacrylamide (CIPAAm) monomer, with a side chain structure similar to *N*-isopropylacrylamide (IPAAm). Hydrogels containing more than 10 mol % CIPAAm exhibit large and sensitive volume phase transitions in response to temperature changes. These volume phase transition temperatures were nearly identical to that seen for IPAAm homopolymer gels. This is in contrast to IPAAm−acrylic acid (AAc) copolymer gels whose phase transition temperatures increase with reduced magnitudes of phase transitions with increasing AAc content. Moreover, volume phase transition temperatures and transition magnitudes for IPAAm−CIPAAm gels were not influenced by solution pH, which significantly influences the IPAAm−AAc gel. These results indicate that IPAAm−CIPAAm gels maintain their hydrophobic aggregation forces without disruption by ionized or hydrogen-bonded carboxyl groups. Because of the common carboxyl functionality and the noted apparent differences between the structures of CIPAAm and AAc monomers, differences in respective gel behaviors were rationalized to result from the structural analogy of CIPAAm's isopropylamide side chain groups with those of IPAAm. We therefore propose that maintaining alignment of isopropylamide side chains within the copolymer facilitates introduction of large amounts of functional groups into IPAAm copolymer gels without loss of phase transition behavior. The new monomer, CIPAAm, should prove useful to introduce functional carboxyl groups into temperature-responsive IPAAm hydrogels while maintaining their intrinsic temperature-sensitive behavior.

Introduction

Recently, significant attention has been directed to exploiting the properties of new stimuli-responsive polymers and hydrogels in a diverse assortment of new technologies including drug delivery,[1−3] chromatographic separations,[4,5] bioconjugation,[6] and cell culture systems.[7] It is well-known that poly(*N*-isopropylacrylamide) (PIPAAm), a temperature-responsive polymer, exhibits a rapid and reversible hydration−dehydration change in response to small temperature cycles around its lower critical solution temperature (LCST; 32 °C)[8] in aqueous media. We have been studying the effects of chemical structure and monomer architecture on the swelling−deswelling behavior of PIPAAm hydrogels comprising IPAAm homopolymers and copolymers and have developed temperature-responsive hydrogels of PIPAAm containing mobile PIPAAm grafted side chains with improved extreme temperature responsiveness.[9,10] Unhindered temperature-sensitive grafted polymer side chains undergo more rapid dehydration in response to small temperature increases due to their freely mobile ends. When these grafted chains readily form hydrophobic aggregates above the LCST, the polymer backbone network is prompted to also shrink rapidly due to hydrophobic sites created inside the polymer network. Hence, water is rapidly expelled from the gel with collapse (phase transition).

To develop new intelligent hydrogels with other properties, many researchers have tried to introduce functional groups into PIPAAm hydrogels by copolymerization with functional monomers such as acrylic acid (AAc) or other ionizable comonomers.[11,12] However, in ionized IPAAm copolymer gels, the volume phase transition temperature shifts to a higher temperature, and the sensitivity of its temperature response is also reduced in aqueous media with increasing AAc content.[11,13−15] This might be attributed to the combined ability of ionic comonomer residues to break PIPAAm chain sequences into short, uncooperative network segments, and the imposed excess hydration and electrostatic repulsion of ionized monomers such as AAc weakens thermally driven hydrophobic aggregation of collapsing PIPAAm networks.

Therefore, we pursued a strategy to maintain the structural continuity of isopropylamide-like side groups in PIPAAm chains to retain more sensitive phase transitions. Since the main mechanism proposed for thermally induced phase separation involves the release of hydrating waters clustered around hydrophobic isopropyl groups, retention of such strucuture, despite the presence of ionizable groups, was hypothesized to be important to hydration−rehydration behavior in these systems. Urry and co-workers reported that folding of protein-based copolymers comprising repeating peptide sequences results from changes in water cluster structure surrounding hydrophobic amino acid residues.[16] We supposed that hydrophilic AAc comonomers interfere with NIPAAm hydration water cluster structure, and therefore, introduction of AAc into PIPAAm interfered

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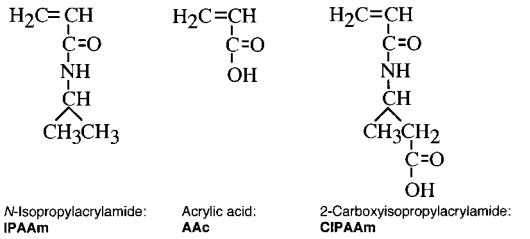

Figure 1. Chemical structures for IPAAm, AAc, and CIPAAm monomers.

with aggregation of the main chains involved with phase transitions.

We have already reported rapid deswelling of PIPAAm gels grafted with hydrophilic poly(ethylene oxide) (PEO) side chains.[17] In contrast with ionized poly(IPAAm-*co*-AAc) gels, nonionized poly(IPAAm-*g*-PEO) gels maintain long sequences of PIPAAm in their network together with larger amounts of hydrophilic moieties grafted into the PIPAAm network without electrostatic interference. Therefore, PEO-grafted PIPAAm gels exhibited abrupt, large volume phase transitions when equally hydrophilic AAc-containing IPAAm gels could not.

On this basis, we have newly synthesized a functional monomer, 2-carboxyisopropylacrylamide (CIPAAm), whose structure is shown in Figure 1[18] together with IPAAm and AAc. CIPAAm contains both the isopropylamide group similar to IPAAm and a carboxyl side group similar to AAc. Moreover, the carboxyl group of CIPAAm is bonded through the isopropyl group, while that of AAc is directly bonded to acrylate group. This design was introduced so that IPAAm copolymer gels with CIPAAm might exhibit strong chain aggregability as this copolymer maintains continuous isopropylamide groups as in the IPAAm homopolymer.

We have already reported that linear, soluble poly(IPAAm-*co*-CIPAAm) containing as much as 10 mol % CIPAAm exhibits a very sensitive phase transition in response to small temperature changes. Also, these LCSTs were not shifted by the introduction of CIPAAm into IPAAm in PBS.[18] Additionally, the LCSTs and sensitivity of the phase transition in linear poly(IPAAm-*co*-CIPAAm) were pH-independent. By contrast, in poly(IPAAm-*co*-AAc) copolymers and other ionic IPAAm copolmer gels,[11] the sensitivity of phase transition and LCSTs were considerably influenced by AAc content, solution pH, and ionic strength.

In this study, we have extended these concepts to the synthesis of new intelligent hydrogels comprising these monomers. The temperature response of these IPAAm-based hydrogels containing large amounts of carboxyl groups introduced through CIPAAm copolymerization with a cross-linking agent were retained. Moreover, their equilibrium swelling ratios were measured and exhibit distinctly different behavior than analogous IPAAm−AAc gels.

Experimental Section

Materials. *N*-Isopropylacrylamide (IPAAm) was kindly provided from Kojin Co., Tokyo, Japan, and purified by recrystallization from *n*-hexane. Acrylic acid (AAc) was distilled under reduced pressure. *N,N,N′,N′*-Tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and *N,N′*-methylenebis(acrylamide) (MBAAm) were purchased from Kanto Chemical Co., Ltd., Tokyo, and used as received.

Synthesis of 2-Carboxyisopropylacrylamide (CIPAAm). 2-Carboxyisopropylacrylamide (CIPAAm) was synthesized as

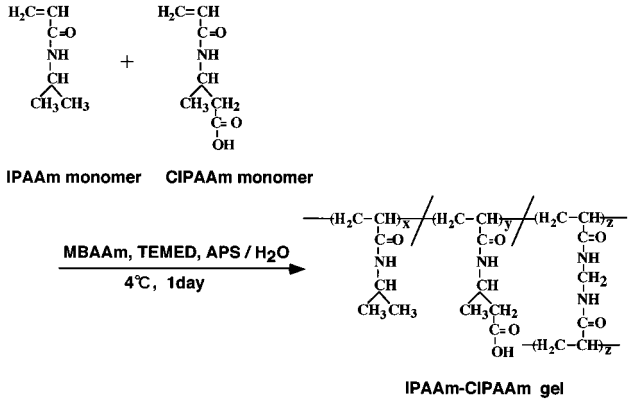

Figure 2. Synthetic representation of IPAAm−CIPAAm gel.

previously described.[18] Briefly, DL-3-aminobutyric acid was esterified using benzyl alcohol. Acryloyl chloride was reacted with DL-3-aminobutyric acid benzyl ester in the presence of triethylamine. CIPAAm was obtained by hydrolysis of benzyl ester using sodium hydroxide aqueous solution. Protonation of carboxyl groups was carried out using an excess amount of hydrochloric acid.

Gel Synthesis. To synthesize gels of small size, IPAAm, CIPAAm, MBAAm, and TEMED were all dissolved in distilled water, and the solution was transferred to a test tube and bubbled with dry nitrogen gas for 15 min to remove oxygen. APS was added to this solution, and then clean glass capillaries of 300 mm i.d. were set into this solution. Spontaneous wetting pulled the solution into the capillaries. The solution was kept at 4 °C for 1 day for polymerization and spontaneous gelation. The synthetic procedure is illustrated in Figure 2. Poly(IPAAm-*co*-CIPAAm) gels are abbreviated as IPAAm−CIPAAm (*X*) gel, where *X* is the mole percent of CIPAAm in the feed. Poly(IPAAm-*co*-AAc) gels and PIPAAm homopolymer gel were synthesized by identical methods and are abbreviated as IPAAm−AAc (*X*) gel and PIPAAm gel, respectively, to denote their respective compositions. After gelation was complete, gels with 300 mm diameter were removed from glass capillaries and cut into small fragments and immersed in pure cold water to remove unreacted compounds for 1 day. Gels were then set into glass capillaries of 1.34 mm diameter filled with phosphate buffer solution with various pH and ionic strength conditions and sealed.

Measurements of Equilibrium Swelling Ratios of the Gels. Equilibrium swelling ratio, $(d/d_0)^3$, was defined as the ratio of gel diameter in equilibrium swollen state to the initial gel diameter (300 μm). The diameter of the gel, d, was measured as a function of temperature using a microscope with a calibrated scale.

Results and Discussion

IPAAm−CIPAAm Copolymerization. As described above, poly(IPAAm-*co*-AAc) random copolymers exhibit higher LCSTs with increasing AAc content resulting from decreased hydrophobic aggregation forces above the LCST. This is presumably because the introduction of polar, ionizable carboxyl groups into PIPAAm chains increases polymer hydration, simultaneously disrupts the continuity of the isopropylamide groups of PIPAAm, and introduces long-range electrostatic repulsive forces. We therefore focused on maintaining a contiguous repeating isopropylamide side chain strucutre in the copolymer. The effect of this structure should be manifested by similar temperature sensitivity as an IPAAm homopolymer. We had already designed the 2-carboxyisopropylacrylamide (CIPAAm) monomer for this purpose and characterized linear, soluble IPAAm−CIPAAm copolymers.[18] In these aqueous solutions, LCSTs and temperature sensitivity of poly(IPAAm-*co*-CIPAAm)

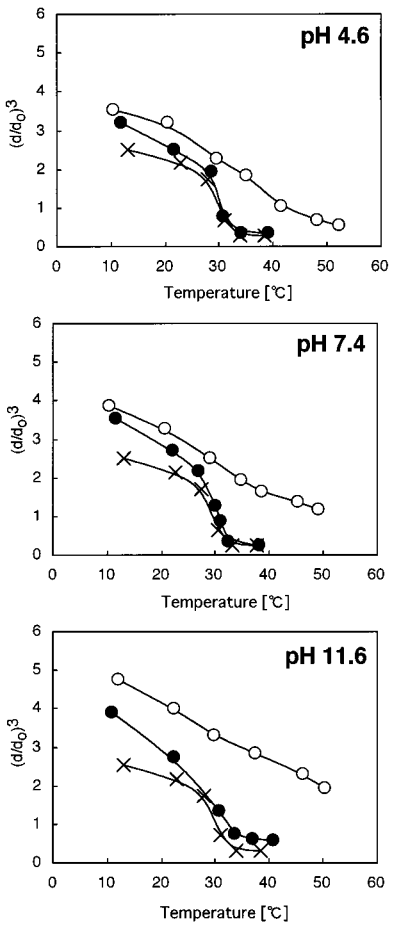

Figure 3. Equilibrium swelling ratios for PIPAAm gel, IPAAm-AAc (5) gel, and IPAAm−CIPAAm (5) gel as a function of temperature at various pH in PBS: ×, PIPAAm gel; ○, IPAAm−AAc (5) gel; ●, IPAAm−CIPAAm (5) gel.

were independent of both carboxyl group content and solution pH, consistent with our design hypothesis and, importantly, distinctly different from that of poly(IPAAm-*co*-AAc) analogous copolymer systems.

Hydrogels prepared from poly(IPAAm-*co*-CIPAAm) copolymerization exhibit equally interesting and analogous properties as their soluble, linear system as determined by equilibrium swelling ratios of the gels as a function of temperature, pH, and ionic strength.

Effect of pH. Figure 3 shows volume phase transition profiles for the PIPAAm gel, IPAAm-AAc (5) gel, and IPAAm−CIPAAm (5) gel in pH 4.6, 7.4, and 11.6 phosphate buffer solutions (0.15 M), respectively. No pH effect was observed for the PIPAAm gel because it contains no ionizable groups. In contrast, the equilibrium swelling ratio of IPAAm−AAc (5) gel increased below its observed LCST, and the sensitivity of its temperature-responsive behavior was reduced with increasing pH. The acidic groups cause swelling primarily via Donnan ion exclusion, followed by swelling changes by electrostatic repulsion.[11] Repulsive forces between AAc carboxylate anions increase polymer chain−chain repulsion, and their polarity and charge increase chain hydration, both of which restrict temperature-induced hydrophobic network aggregation.[19] In the IPAAm−CIPAAm (5) gel, equilibrium swelling ratios increased with increasing pH at low temperature and are higher than that of the PIPAAm gel because of Donnan effects. However, the phase transition temperature for the IPAAm−CIPAAm (5) gel was nearly identical to that of the PIPAAm gel, and its transition sensitivity behaves as the PIPAAm gel: the volume phase transition occurred over a very narrow temperature range. Using pH titration, the pK_a for poly(IPAAm-*co*-CIPAAm) (CIPAAm content 4.8 mol %) was 6.2 while that of poly(IPAAm-*co*-AAc) (AAc content 4.6 mol %) was about 5.0 at 10 °C. Therefore, fewer carboxyl groups of IPAAm−CIPAAm (5) gel were dissociated compared to the IPAAm−AAc (5) gel under these swelling conditions. We propose that maintenance of a continuous sequence of isopropylamide side groups within the IPAAm−CIPAAm (5) gel suppressed dissociation of carboxyl groups, retaining the desired hydrophobic environment around the collapsing polymer chains. Moreover, this continuous isopropylamide group sequence in the IPAAm−CIPAAm (5) gel was maintained without interfering with near-normal PIPAAm chain aggregation upon dehydration. Hoffman's group has synthesized graft copolymers comprising grafted chains of a temperature-sensitive PIPAAm bound to a main chain of poly(acrylic acid).[20] In contrast to random IPAAm−AAc copolymers, the temperature-sensitive properties of this PAAc grafted with PIPAAm were retained over a wide range of conditions because individual, long PIPAAm graft segments in these types of copolymers tend to behave independently of the PAAc backbone. These results support the importance of continuous isopropylamide groups to produce abrupt chain dehydration and their resulting aggregation.

Effect of Ionic Strength. The influence of medium (buffer) ionic strength on the phase transitions of PIPAAm gel, IPAAm-AAc (5) gel, and IPAAm−CIPAAm (5) gel was studied by adjusting the ionic strength with NaCl (at constant pH 7.4). Figure 4 shows that equilibrium swelling ratios of each gel decreased with increasing of ionic strength and simultaneously LCSTs shifted to low temperature. In general, increased electrolyte concentration tends to both screen polymer charge repulsion as well as decrease polymer solubility due to salting-out effects, even though there are a few exceptional salts.[11,15,21] The sensitivity of the volumetric phase transition of the IPAAm−AAc (5) gel was reduced with decreasing ionic strength. In particular, sensitivity of volume phase transition of IPAAm−AAc (5) gel at 0.05 M was greatly reduced. Significant amounts of carboxyl groups dissociate at low ionic strength conditions, preventing copolymer gel aggregation with increasing temperature. However, the sensitivities of volume phase transition of IPAAm−CIPAAm (5) gel and PIPAAm gel were not influenced by ionic strength. In the former case, dissociation of carboxyl groups within the IPAAm−CIPAAm (5) gel do not affect copolymer gel aggregation as these carboxyl groups are displaced from the dehydrating polymer backbone, similar to previous work on other grafted systems.[20]

Effect of Comonomer Content. Figure 5 shows the volume phase transition profiles for IPAAm−AAc and IPAAm−CIPAAm gels in phosphate buffer solutions (at constant ionic strength of 0.15 M and pH 7.4) as a function of comonomer content. The LCSTs for the IPAAm−AAc gels were found shift to higher temperature with considerably broader volume phase transition with increasing AAc content. This is presumably a result of the large content of carboxylate of AAc that suppresses the opposing hydrophobic copolymer aggregation forces. In contrast, the IPAAm−CIPAAm (10) gel and IPAAm−CIPAAm (20) gel showed very sharp

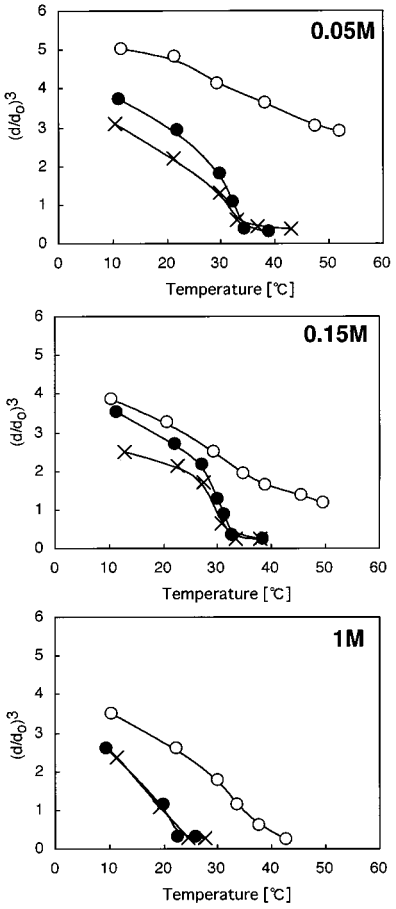

Figure 4. Equilibrium swelling ratios for PIPAAm gel, IPAAm−AAc (5) gel, and IPAAm−CIPAAm (5) gel as a function of temperature in various ionic strength in PBS: ×, PIPAAm gel; ○, IPAAm−AAc (5) gel; ●, IPAAm−CIPAAm (5) gel.

volume phase transitions in response to temperature change, and their transition temperatures were nearly identical to that of the IPAAm homopolymer gel even though they contain a large carboxyl group content. IPAAm−AAc (3) gel and IPAAm−AAc (5) gel LCSTs shifted to higher temperatures, and their sensitivity of volume phase transition was reduced. Hence, the volume phase transition of IPAAm−CIPAAm gel appeared to be consistently sharp and not influenced by CIPAAm comonomer content.

Structual Design of These Hydrogels. The thermally sensitive hydrophilic/hydrophobic balance of PIPAAm segments is a direct function of this monomer chemistry and resulting hydration. This unique structure has long been proposed to contribute to abrupt dehydration observed with increasing temperature. Therefore, introduction of carboxyl groups (such as AAc) into PIPAAm presumably disturbs this hydrophilic/hydrophobic balance in both chemical and structural ways, producing excess hydration and electrostatic repulsion that hinder polymer aggregation above the LCST. IPAAm−CIPAAm gels and linear polymers both exhibit sensitive dehydration with increasing temperature. Carboxyl groups in CIPAAm bond through its isopropylamide side group, suggesting the proximity of the charged group along the dehyrating chain in IPAAm−AAc copolymers as well as interrupted backbone isopropylacrylamide structure resulting from copolymerization both preculde effective dehydration. Possibly, extended alignment of the isopropylamide side chain

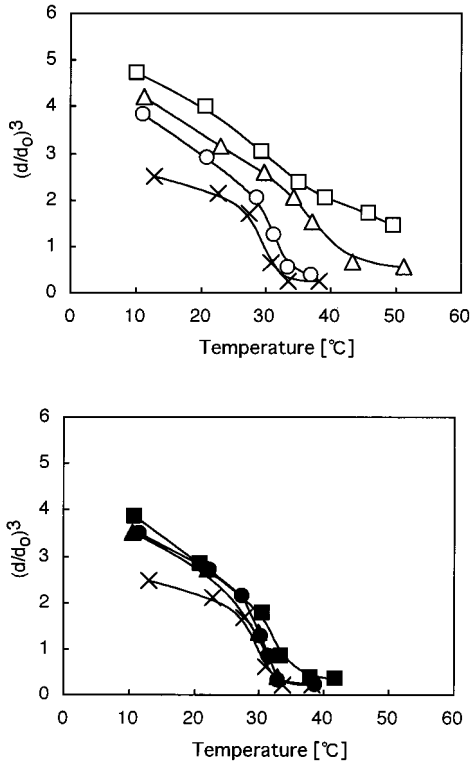

Figure 5. Equilibrium swelling ratios forIPAAm−AAc gel and IPAAm−CIPAAm gel at various compositions as a function of temperature in pH 7.4 PBS: ×, PIPAAm gel; ○, IPAAm−AAc (1) gel; △, IPAAm−AAc (3) gel; □, IPAAm−AAc (5) gel; ●, IPAAm−CIPAAm (5) gel; ▲, IPAAm−CIPAAm (10) gel; ■, IPAAm−CIPAAm (20) gel.

groups, despite the presence of a remote charged carboxyl group in CIPAAm copolymers, maintains water cluster structure similar to that of the IPAAm homopolymer. As a result, utilization of CIPAAm makes it possible to introduce large amounts of useful carboxyl functional side groups into PIPAAm gels while retaining sensitive temperature-responsive behavior.

With these useful properties, IPAAm−CIPAAm hydrogel applications can now be readily extended into further bioengineering fields. This hydrogel should have the potential for new temperature-modulated membranes and bioreactors containing CIPAAm-immobilized species, hybrid devices containing electrochemical or optical moieties, and controlled release devices involving bioactive molecules.

Conclusion

In this paper, new temperature-responsive hydrogels containing carboxyl groups were synthesized by copolymerization of IPAAm and CIPAAm. From equilibrium swelling ratio measurements, IPAAm−CIPAAm gels showed very sensitive volume phase transitions, and their LCSTs were comparable to that of IPAAm homopolymer gels. This behavior was independent of CIPAAm content and solution pH (side chain charge). By contrast, random IPAAm−AAc gel LCSTs shifted to higher temperatures, and the sensitivities of their volume phase transitions decreased with increasing solution pH and AAc content. At low ionic strength, IPAAm−CIPAAm gels showed sensitive volume phase transition while that for the IPAAm−AAc gel was greatly reduced. These results indicate that carboxyl groups in IPAAm−CIPAAm gels behave differently from

those in IPAAm−AAc gels. We propose that the IPAAm−CIPAAm gel maintains the required, strong hydrophobic aggregation forces within PIPAAm network necessary to produce effective collapse, even with the introduction of large amounts of carboxyl groups. We rationalize that this is due to retention of a continuous chain of isopropylamide groups similar to a IPAAm homopolymer in the IPAAm−CIPAAm gel. Because relatively small amounts of AAc comonomer decrease the hydrophobic aggregation forces in PIPAAm gels due both to charge−charge repulsion and to disruption of water cluster formation around the isopropylamide side groups, rearrangement of this charged species away from the PIPAAm backbone and remotely in the side chain (e.g., in CIPAAm gels) appears to change the gel hydration/dehydration behavior without influencing gel hydrophilic/hydrophobic balance.

Using comonomer CIPAAm instead of more traditional AAc, it is possible to introduce large quantities of functional side chain carboxyl groups into IPAAm hydrogels while retaining sensitive gel phase transition response to temperature.

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