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In situ formation of thermosensitive P(NIPAAm-*co*-GMA)/PEI hydrogels

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ABSTRACT

A strategy for in situ chemical gelation of poly(*N*-isopropylacrylamide-*co*-glycidyl methacrylate)/polyethylenimine (P(NIPAAm-*co*-GMA)/PEI) polymers has been demonstrated. Two kinds of P(NIPAAm-*co*-GMA) with epoxy pendant groups were prepared. When the solution of P(NIPAAm-*co*-GMA) was mixed with branched polyethylenimine (PEI, M_w 800), the cross-linking between the epoxy functional groups and amines, a type of nucleophilic substitution reaction occurred. The corresponding gelation process was confirmed via rheology. The in situ formed hydrogels were studied via scanning electric microscopy (SEM) and the equilibrium swelling ratio, swelling kinetics, and temperature response kinetics were examined. The strategy described here presents a potential alternative to the traditional synthesis techniques for the in situ formation of thermosensitive hydrogels.

1. Introduction

Hydrogels are a class of materials that can contain a lot of water in their swollen network structure without dissolving [1–3]. Because of the similarity between the highly hydrated three-dimensional matrix of hydrogels and hydrated body tissues, as well as their high biocompatibility, hydrogels have been widely explored for biomedical applications ranging from drug controlled release [4] to tissue engineering [5].

Poly(*N*-isopropylacrylamide) (PNIPAAm) and its derivatives are the most popular thermosensitive polymers. PNIPAAm exhibits phase separation at the lower critical solution temperature (LCST) of ~32 °C in aqueous solution [6,7]. Below this temperature, PNIPAAm is hydrophilic and exists in individual chains with a coil conformation; while above the LCST, it undergoes a sharp coil-to-globule transition to form inter- and intra-chain associations, resulting in hydrophobic aggregation and deposition from the aqueous solution [8–11]. Because of this unique property, the use of hydrogels based on PNIPAAm in many biomedical applications, including artificial organs [12], actuators [13], and on–off switches [14] has been explored. The most common synthesis method for cross-linked PNIPAAm-based hydrogel materials involves the free radical polymerization of water-soluble *N*-isopropylacrylamide (NIPAAm) in the presence of cross-linker, such as *N*,*N*′-methylenebisacrylamide (MBAAm) [15–18] or poly(ethyleneglycol)-diacrylate [19–21]. However, the application of these cross-linking methods is generally accompanied by a large degree of intramolecular cross-link formation [22]. Recently, other chemoselective cross-linking reactions initiated by simple mixing of polymer component solutions have been employed for the hydrogel formation [23–26]. For example, the coupling reaction between an aldehyde and a hydrazide [23] or cysteine1,2-aminothiol group [26] has been used for hydrogel formation. More recently, Michael addition of thiols to acrylates or vinyl sulfones was applied for PNIPAAm hydrogel in situ formation [27,28]. These reactions can be carried out at physiological temperature and pH. A major drawback, however, in using thiol-functionalized compounds is their sensitivity to oxidation [22].

At present, nucleophilic substitution reactions between the epoxy functional groups and amines have been widely used for the covalent binding of proteins [29], the synthesis of grafted polymers [30], and the preparation of core–shell nanoparticles [31]. Numerous reports demonstrate that such nucleophilic substitution reactions have several advantages including mild reaction conditions, high specificity, and near-perfect fidelity in the presence of most functional groups. One may therefore incorporate the epoxy functional groups into PNIPAAm for the in situ formation of thermosensitive hydrogels. However, up to now, there have been very few reports on in situ thermosensitive hydrogel formation using such a nucleophilic substitution reaction, though other non-thermosensitive hydrogels formed by this reaction have been described [32,33]. Thermosensitive hydrogels have attracted extensive interest because of their potential for applications in many fields, including protein-ligand recognition [34], the generation of on–off switches for modulated drug delivery [4], and immobilization of enzymes [35]. In this study, two kinds of P(NIPAAm-*co*-GMA) with epoxy pendant groups were prepared. When

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the solution of P(NIPAAm-*co*-GMA) was mixed with branched polyethylenimine (PEI, M_w 800), the cross-linking between the epoxy functional groups and amines appeared. In comparison with other methods for in situ hydrogel formation, the strategy described here could overcome several drawbacks such as the sensitivity of thiol-functionalized compounds to oxidation in Michael addition reaction. Simultaneously, the superiorities of such nucleophilic substitution reactions would provide several advantages for in situ hydrogel formation, including mild gelation conditions, high chemoselectivity, and fast cross-linking, which would affect the macroscopic properties of the resulted hydrogels. This strategy presents a potential alternative to traditional synthesis techniques for the in situ formation of thermosensitive hydrogels.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAAm) was purchased from ACROS (USA) and used as received. Branched polyethylenimine (PEI, M_w 800) was provided by Sigma–Aldrich and also used as received. Glycidyl methacrylate (GMA), *N,N′*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were obtained from Shanghai Reagent Chemical Co. (China) and distilled before use. *N,N′*-Azobisisobutyronitrile (AIBN) was provided by Shanghai Reagent Chemical Co. (China) and recrystallized from ethanol. All other reagents and solvents were of analytical grade and used without further purification.

2.2. Preparation of P(NIPAAm-co-GMA)

NIPAAm and GMA with different molar ratios (12:1 for **1** and 9:1 for **2**), and AIBN as an initiator (0.5 mol% based on the total amount of monomers), were thoroughly dissolved in dry DMF. After degassing with nitrogen for 30 min, the polymerization of the mixture was carried out at 70 °C for 24 h under nitrogen. Products **1** and **2** were collected after precipitation in chilled diethyl ether and subsequently dried under vacuum. The detailed feed composition is summarized in Table 1.

2.3. GPC measurements

The number- and weight-average molecular weights (M_n and M_w, respectively) of the polymers were determined by a gel permeation chromatographic (GPC) system equipped with a Waters 2690D separations module and a Waters 2410 refractive index detector. THF was used as the eluent at a flow rate of 0.3 mL/min. Waters Millennium Module software was used to calculate molecular weight on the basis of a universal calibration curve generated by a polystyrene standard with a narrow molecular weight distribution.

2.4. FT-IR measurements

The polymers were analyzed using a FT-IR (Perkin–Elmer Spectrum One, USA) spectrophotometer. Before the measurements, the samples were pressed into potassium bromide (KBr) pellets.

2.5. ^{1}H NMR measurements

The ^{1}H NMR spectra of polymers were recorded using a Mercury VX-300 spectrometer at 300 MHz (Varian, USA) by using $CDCl_3$ as a solvent and TMS as an internal standard.

2.6. In situ hydrogel formation

The specified amounts of P(NIPAAm-*co*-GMA) and branched PEI were dissolved in DMSO to form a homogenous solution. After gelation at 30 °C for 24 h, the formed hydrogel was taken out and immersed in distilled water for 7 days at room temperature (22 °C). During this period, the distilled water was refreshed every several hours in order to leach out the unreacted chemicals. After that, the hydrogel was cut into disc-like pieces ~10 mm in diameter and 4 mm in thickness for characterization. The detailed feed composition is listed in Table 2.

2.7. Oscillatory rheology

Rheology experiments were performed at 30 °C on an ARES-RFS III rheometer (TA Instruments, USA). Briefly, 8 mL P(NIPAAm-*co*-GMA) and branched PEI solution was prepared at room temperature as described above, then quickly transferred to the rheometer. The storage modulus (G′) and loss modulus (G″) were recorded to study the viscoelastic properties of the in situ formed hydrogels.

2.8. Interior morphology

The swollen hydrogel samples, after reaching an equilibrium state in the distilled water at room temperature, were quickly frozen in liquid nitrogen and then freeze-dried in a Freeze Drier (Labconco CA, USA) under vacuum at −45 °C for 3 days. The freeze-dried samples were then fractured carefully in liquid nitrogen and the interior morphology of hydrogel samples was studied using a scanning electron microscope (SEM, FEI-QUANTA 200, Holland). Before SEM observations, the hydrogel specimens were coated with gold for 7 min.

2.9. Temperature dependence of swelling ratio

The gravimetric method was employed to study the temperature dependence of the swelling ratio of the hydrogels. The samples were equilibrated in distilled water at a temperature ranging from 5 to 60 °C. The samples were allowed to swell in the distilled water for 24 h at each predetermined temperature by a temperature-controlled water bath. After 24 h of immersion in the distilled water, the samples were weighed after wiping off the excess water on the surfaces by moistened filter paper. Each sample was measured three times and the average value of three measurements was taken. After this weight measurement, the samples were re-equilibrated in distilled water at another predetermined temperature and then their wet weights were determined as mentioned above. The dry weight of each sample was obtained after drying

Table 1
Feed compositions of the P(NIPAAm-*co*-GMA) copolymers.

		Monomers (mmol)		M_n[a]	Yield (%)	D (M_w/M_n)	MR[b]
		NIPAAm	GMA				
P(NIPAAm-*co*-GMA)	1	40	3.33	18,700	89.2	1.77	12.1:1
	2	40	4.52	18,400	96	1.86	9.4:1

[a] Determined by GPC measurement.
[b] Molar ratio of NIPAAm units to GMA units in the polymeric chain calculated from the ^{1}H NMR measurements.

Table 2
Feed compositions of the in situ formed P(NIPAAm-*co*-GMA)/PEI hydrogels.

	Gel1	Gel2	Gel3	Gel4
P(NIPAAm-*co*-GMA) (mg)	300 (**1**)	300 (**2**)	300 (**1**)	300 (**2**)
PEI (mg)	24	24	12	12
DMSO (mL)	3	3	3	3