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Facile preparation of degradable thermoresponsive polymers as biomaterials: Thermoresponsive polymers prepared by radical polymerization degrade to water-soluble oligomers

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ABSTRACT

A novel thermoresponsive and biodegradable polymer, poly(MDO-*co*-HEA), was prepared by radical copolymerization of 2-methylene-1,3-dioxepane (MDO) and a hydrophilic vinyl monomer, 2-hydroxyethyl acrylate (HEA), in dimethylsulfoxide (DMSO), varying feed monomer compositions. Poly(MDO-*co*-HEA) showed lower critical solution temperature (LCST)-type phase separation in aqueous medium, forming coacervate droplets into which low molecular weight hydrophobic molecules could be loaded above the LCST. The LCST could be controlled not only by the chemical compositions of the hydrophobic MDO and hydrophilic HEA in the polymer chains, but also by ion and polymer concentration. Degradation tests in aqueous media indicated that poly(MDO-*co*-HEA) was converted into hydrophilic oligomers by hydrolysis of the ester groups in the polymer backbone. The facile preparation poly(MDO-*co*-HEA) are valuable for use in functional biomedical materials, such as base of drug delivery carrier and cell culture scaffold instead of non-degradable stimuli-responsive polymer.

1. Introduction

Stimuli-responsive polymers have been used in many fields, including biomedical science [1,2], because they are able to change their solubilities in aqueous media in response to external stimuli such as temperature [3–5,10–22], pH [6,7], and light [8,9]. The thermoresponsive polymer poly(*N*-isopropylacrylamide) (PNIPAAm) has been especially widely studied as a biomaterial. PNIPAAm shows phase separation in water above 32 °C, which is referred to as the lower critical solution temperature (LCST) [10–17]. Below the LCST, PNIPAAm is hydrated and soluble in water, existing in a random coil conformation. However, above the LCST PNIPAAm becomes hydrophobic due to the dehydration of the isopropyl groups on the side chains, resulting in the adoption of a globular state [15–18]. PNIPAAm chains thus show a conformational change from random coils to globules, resulting in a so-called liquid-solid phase separation. Several biomedical applications have been reported that utilize the thermoresponsive properties of PNIPAAm in the fields of diagnosis [10,11], drug delivery [11,12], cell scaffolding [13], and thermoresponsive chromatography [15].

Biodegradability is often required when using polymers as biomaterials in the living body because this allows materials to be metabolized and excreted from the body after treatment. Many researchers have focused on thermoresponsive polyesters and poly(amino acid) derivatives, including amphiphilic polyesters based on poly(ε-caprolactone) (PCL) [23,24], poly(lactic acid) (PLA) [25], and elastin-like polypeptide (ELP) [26]. It is difficult to functionalize these polymers further, although functionalization is often carried out by post-modification methods such as click reactions [27]. Synthesis of biodegradable polymers is carried out not only using ionic ring-opening polymerization and/or amino acid synthesis, but also via radical polymerization. 2-Methylene-1,3-dioxepane (MDO) is a well-known hydrophobic cyclic ketene acetal monomer [27–33] that can undergo radical homopolymerization as well as radical copolymerization with other vinyl monomers such as NIPAAm [14], oligo (ethylene glycol) vinyl acetate ester [30] or glycidyl methacrylate (GMA) [32]. However, these copolymers remain their thermoresponsive properties or form hydrophobic oligomers after degradation. Such oligomers involve a risk of aggregation in the body due to hydrophobic interactions. Hence, it is necessary to develop polymers that are able to form

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hydrophilic oligomers after degradation.

Herein, we focused on the chemical structure of thermoresponsive polymers for the preparation of biodegradable thermoresponsive polymers. LCST-type phase separation is attributed to a balance hydrophilicity and hydrophobicity in polymers [5,19–22]. Thermoresponsive polymers are often synthesized by copolymerization of pairs of hydrophilic and hydrophobic monomers, with poly[(*N*-vinylamide)-*co*-(vinyl acetate)] [19] and poly[(*N*,*N*-dimethylacrylamide)-*co*-(*N*-phenylacrylamide)] [20] being typical examples of thermoresponsive polymers that show LCST-type thermoresponsive behavior in aqueous media. In this study, we prepared a biodegradable polymer showing LCST-type thermoresponsive behavior, poly(MDO-*co*-2-hydroxyethyl acrylate), by radical copolymerization using MDO as the hydrophobic monomer and 2-hydroxyethyl acrylate (HEA) as a hydrophilic vinyl monomer. The LCSTs of the copolymers were successfully regulated by altering the monomer feed compositions. Above the LCST, the copolymer formed coacervate droplets that were able to envelop a low molecular weight hydrophobic substance in an aqueous medium. Degradability was investigated at 45 °C under alkaline aqueous conditions. After hydrolysis, Poly(MDO-*co*-HEA) change to hydrophilic oligomer.

2. Experimental

2.1. Materials

Chloroacetaldehyde dimethylacetal was purchased from Sigma-Aldrich (USA). Potassium *t*-butoxide (*t*-BuOK), 1,4-butanediol, Dowex 50 (H^+), tetrahydrofuran (THF), 2,2′-azobis (4-methoxy-2,4-dimethylvaleronitrile) (V-70), rhodamine B, dimethyl sulfoxide (DMSO, and 2-hydroxyethylacrylate (HEA) were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). DMSO was distilled under reduced pressure before use (0.5 kPa, 95.0 °C). 2-Methylene-1,3-dioxepane (MDO) was prepared by a two-step reaction according to previous reports [29,31]. In brief, 2-chloromethyl-1,3-dioxepane (CDO) was prepared by the reaction of 1,4-butanediol and chloroacetaldehyde dimethylacetal on Dowex 50(H^+). MDO was then obtained from CDO by a dehydrochlorination reaction using *t*-BuOK in THF, followed by purification by distillation under reduced pressure (3.8 kPa, 70 °C, yield: 68%). Preparation of MDO was confirmed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (^{1}H NMR 600 MHz UltraShield, Bruker). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.70–1.80 (s, 4H, -$OCH_2\underline{CH_2}\underline{CH_2}CH_2O$-), 3.48 (s, 2H, CH_2=C), 3.85–4.00 (s, 4H, -OCH_2-).

2.2. Preparation of poly(MDO-co-HEA)

Free-radical ring-opening copolymerization of varying ratios of MDO and HEA was carried out over 24 h in DMSO at 30 °C under nitrogen atmospheres using 2 mol% V-70 as a radical initiator to restrain transesterification reaction [34]. The resulting solutions were dialyzed against methanol for 3 days using Spectra/Por 3 regenerated cellulose membrane tubing (molecular weight cutoff of 3500 Da), and then in water for 5 days before recovery of the products by lyophilization. The chemical structure of poly(MDO-*co*-HEA) was confirmed by ^{1}H NMR spectroscopy. The number average molecular weights and polydispersities of poly(MDO-*co*-HEA) with various compositions were determined by gel permeation chromatography (GPC) at 45 °C using DMF containing 10 mmol L^{-1} LiCl as an eluent. Poly(ethylene glycol)s with polydispersity indexes (PDIs) below 1.01 were used to generate the calibration curve. Differential scanning calorimetry (DSC, EXSTAR 6000, Seiko Instruments, Tokyo, Japan) was used to measure the thermoresponsive properties of aqueous solutions of PNIPAAm and poly(MDO-*co*-HEA) (polymer conc. of 10 wt%). DSC measurements were carried out between 0 and 100 °C at a scanning rate of 2 °C/min.

2.3. Thermoresponsive properties of poly(MDO-co-HEA)

Thermoresponsive properties were measured using the temperature-dependent transmittance changes relating to the turbidities of 1.0 wt% aqueous solutions of poly(MDO-*co*-HEA), as measured by using a UV-vis spectrophotometer (V-630Bio, JASCO, Tokyo, Japan) connected to a Pertier thermostat (ETC-717, OPS-512 TYPE, JASCO) (λ: 500 nm, heating and cooling rates: 1.0 °C/min, respectively). The thermoresponsive properties of the polymer solutions were also investigated using the same apparatus and varying the salt (0–150 mmol L^{-1} NaCl) and polymer (0.125–1.0 wt %) concentrations. The LCST was defined as the temperature at which a polymer solution showed 50% transmittance. Microscopic observations of poly(MDO-*co*-HEA) aqueous solutions were performed on micro-droplets on glass coverslips both below (25 °C) and above (45 °C) the LCST. The temperature of the sample was controlled using a microwarming plate (KM-1, Kitazato Science, Tokyo, Japan). Rhodamine B (1 μg/mL) was used as a model hydrophobic compound in the microscopic observations.

2.4. Degradation of poly(MDO-co-HEA)

Hydrolysis of poly(MDO-*co*-HEA) was performed under alkaline conditions as an accelerated test. For alkaline hydrolysis, 1.0 wt% of poly(MDO-*co*-HEA) was dissolved in a 0.5 mol L^{-1} NaOH aqueous solution, then immersed in a thermostatically controlled water bath at 45 °C for 24 h. The number average molecular weights of degraded poly(MDO-*co*-HEA) samples were determined by GPC. The turbidities of the polymers during hydrolysis above their LCSTs were monitored using a UV-vis spectrophotometer.

3. Results and discussion

3.1. Preparation of poly(MDO-co-HEA)

Poly(MDO-*co*-HEA)s were prepared by the free radical copolymerization of MDO and HEA (Fig. 1a). Fig. 1b shows the ^{1}H NMR spectrum of poly(MDO-*co*-HEA), indicating the characteristic peaks: the methylene protons of the HEA side chains at 3.5–3.6 ppm, and the methylene protons of MDO and HEA at 3.9–4.1 ppm. Data shown in Fig. 1b indicates that MDO and HEA copolymerized in DMSO, and that ester groups were introduced into the polymer backbone as shown in Fig. 1a. The results of the radical copolymerizations are summarized in Table 1. The number average molecular weights were 50,000–60,000 Da and the PDIs were approximately 2. By varying the feed ratio of the MDO component, the amount of MDO in the copolymer could be controlled from 3.2 to 10.9%, respectively.

3.2. Analysis of thermoresponsive behavior

The prepared polymers were dissolved in water. As the copolymers possessed both hydrophilic and hydrophobic segments, their thermoresponsive behaviors were examined. As shown in Fig. 2a, polymer solutions became turbid upon incubation in a water bath at 40 °C. The thermoresponsive solubility changes of poly(MDO-*co*-HEA) samples were then determined by measuring the temperature dependent transmittances of 1.0 wt% aqueous solutions of the polymers at 500 nm between 15 and 45 °C using a UV-vis spectrometer, and the results are shown in Fig. 2b. The