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Thermo-responsive polymer brush-grafted porous polystyrene beads for all-aqueous chromatography

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ABSTRACT

Poly(*N*-isopropylacrylamide) (PIPAAm) brush-grafted porous polystyrene beads with variable grafted polymer densities were prepared using surface-initiated atom transfer radical polymerization (ATRP) for applications in thermo-responsive chromatography. Utilization of these grafted beads as a stationary phase in aqueous chromatographic analysis of insulin provides a graft density-dependent analyte retention behavior. The separations calibration curve on PIPAAm-grafted polystyrene was obtained using pullulan standards and exhibited inflection points attributed to analyte diffusion into bead pores and partitioning into grafted PIPAAm brush surfaces. Presence of these inflection points supports a separation mechanism where insulin penetrates pores in polystyrene beads and hydrophobically interacts with PIPAAm brushes grafted within the pores. Control of PIPAAm brush graft density on polystyrene facilitates effective aqueous phase separation of peptides based on thermally modulated hydrophobic interactions with grafted PIPAAm within stationary phase pores. These results indicated that PIPAAm brush-grafted porous polystyrene beads prepared by surface-initiated ATRP was effective stationary phase of thermo-responsive chromatography for aqueous phase peptide separations.

1. Introduction

Poly(*N*-isopropylacrylamide) (PIPAAm), a popular stimuli-sensitive polymer, exhibits temperature-responsive soluble–insoluble change across its lower critical solution temperature (LCST) at 32 °C in aqueous solution [1]. Its solubility change can be explained by reversible hydration/dehydration of polymer isopropyl side chains, hydrating to expand chains in water below the LCST, while dehydrating to form compact, insoluble conformations above the LCST [1,2]. This intrinsic thermo-responsive property is widely exploited in cell-related biomedical applications, including cell culture substrates [3,4], and tissue engineering for regenerative medicine [5]. Additionally, the property has been exploited in all-aqueous hydrophobic chromatographic separations of peptides and bioactive mixtures [6,7]. This system is highly useful to control both stationary phase function and properties for aqueous mobile phase high performance liquid chromatography (HPLC) by changing only column temperature. Performance advantages include maintenance of biological activity of peptides and proteins, and reduced waste from organic mobile phases commonly used in reversed-phase chromatography.

Nonetheless, silica beads exhibit instability under alkaline conditions, and are best used in neutral pH mobile phases [8]. Experimentally, separation reproducibility decreases with repeated use of the silica-based stationary phases even at neutral condition. Thus, base materials with aqueous stability at neutral-high pH are required for improved aqueous separations reproducibility. We chose highly cross-linked porous polystyrene beads as the base materials of novel thermo-responsive chromatography matrices due to their high stability [9]. However, the use of polystyrene beads for the separation of bioactive compounds would be limited, since strong hydrophobicity of polystyrene causes non-specific adsorption of analytes [10].

In order to use polystyrene beads as improved chromatography stationary phases, we prepared dense PIPAAm brush-grafted porous polystyrene beads by surface-initiated atom transfer radical polymerization (ATRP). Surface-initiated ATRP is an attractive polymer grafting method since it allows for the preparation of dense polymer brushes using surface-immobilized ATRP initiators [11–13]. Recently, some research groups had applied polymer brush-grafted silica beads prepared by ATRP to the stationary phases of reversed-phase chromatography [14,15] and thermo-

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responsive chromatography [16]. Dense polymer brush-grafted beads enhanced the retention of hydrophobic compounds compared to that using sparsely grafted beads [14–16]. These results revealed that modification of dense polymer brush on beads surface remarkably improved the performance of the beads as chromatography stationary phase. In the preparation of PIPAAm-grafted polystyrene beads, surface-initiated ATRP can be a promising technique to restrict non-specific adsorption of bioactive compounds by densely grafting of PIPAAm [17]. Furthermore, chain length [18–20] and densities [21] of grafted polymer products can be regulated by varying the ATRP conditions. In the previous reports of thermo-responsive chromatography using silica beads as the base material, PIPAAm graft length and densities remarkably influence thermo-responsive hydrophobicity changes and the elution behavior of aqueous phase analytes, factors that can be modulated for the effective separation of bioactive compounds in water [16,22].

In the present study, we investigated polymer grafting of PIPAAm on porous polystyrene beads as the novel highly stable stationary phase for thermo-responsive chromatography. Dense PIPAAm brush-grafted polystyrene beads with varying graft amounts were prepared using surface-initiated ATRP. To investigate the appropriate PIPAAm grafting condition for thermo-responsive chromatography stationary phases, the prepared beads were evaluated by chromatographic analysis using standard pullulans and peptides.

2. Experimental

2.1. Materials

N-isopropylacrylamide (IPAAm) was kindly provided by Kohjin (Tokyo, Japan) and purified by recrystallization from *n*-hexane, followed by thorough drying in vacuo at 25 °C. Poly(styrene-divinylbenzene) beads (MCI GEL, CHP5C, average diameter 10 μm; pore size 250 Å; specific surface area, 600 m^2/g) were obtained from Mitsubishi Chemical Corporation (Tokyo). Chloromethyl methyl ether (CME) and dioxane were purchased from Wako Pure Chemicals Industries, Co. Ltd. (Osaka). Zinc(II) chloride in diethyl ether (1 M $ZnCl_2$ solution) was purchased from Sigma Chemicals (St. Louis, MO, USA). Tris(2-aminoethyl)amine was obtained from Acros Organics (Pittsburgh, PA). Formaldehyde, formic acid, sodium hydroxide, chloroform, and anhydrous magnesium sulfate were purchased from Wako Chemicals. Copper(I) chloride (CuCl), copper(II) chloride ($CuCl_2$), ethylendiamine-*N*,*N*,*N*′,*N*′-tetraacetic acid disodium salt dehydrate (EDTA·2Na), dehydrated 2-propanol, methanol, and acetone were obtained from Wako Chemicals. Phosphate buffer powder (1/15 mol/l, pH 7.0) was purchased from Wako Chemicals. Insulin chain A (oxidized, ammonium salt) from bovine insulin, insulin chain B (oxidized) from bovine insulin, and insulin from bovine pancreas were obtained from Sigma. Glucose was purchased from Wako Chemicals. Standard pullulans (M_w 1300–788,000) were obtained from Showa Denko K.K. (Tokyo).

2.2. Preparation of ATRP initiator-immobilized polystyrene beads

Chloromethylated polystyrene beads, immobilized ATRP-initiating group, were prepared by Friedel-Crafts reaction as shown in Fig. 1(a) [23]. Polystyrene beads (5.0 g) were placed into a cleaned three-neck flask, followed by the addition of predetermined amount of CME at 0 °C under nitrogen atmosphere. We treated CME use with a protective face mask to prevent inhalation since CME is a known carcinogen and via inhalation can cause pneumonia or lung cancer. This suspension was stirred at 0 °C for 2 h to swell beads, followed by the addition of $ZnCl_2$. The reaction then proceeded at 30 or 40 °C under continuous stirring for predetermined times. The reaction mixture gradually became a red color. Dioxane was then added to bleach the red color from the reaction mixture. Chloromethylated beads were filtered and rinsed repeatedly with dioxane and acetone, then dried at 50 °C for 3 h under vacuum. ATRP initiator-immobilized polystyrene beads are abbreviated as CM-*X* where *X* is the amount of modified chloromethyl units in μmol/m^2.

2.3. Preparation of PIPAAm brush grafts on porous polystyrene beads

PIPAAm brush-grafted polystyrene beads were prepared by surface-initiated ATRP on initiator-immobilized polystyrene beads in 2-propanol as shown in Fig. 1 (b). Tris(2-(dimethylamino)ethyl)amine (Me_6TREN) as an ATRP ligand was synthesized using a previously reported method [24]. IPAAm monomer was dissolved in dried 2-propanol to a set initial con-

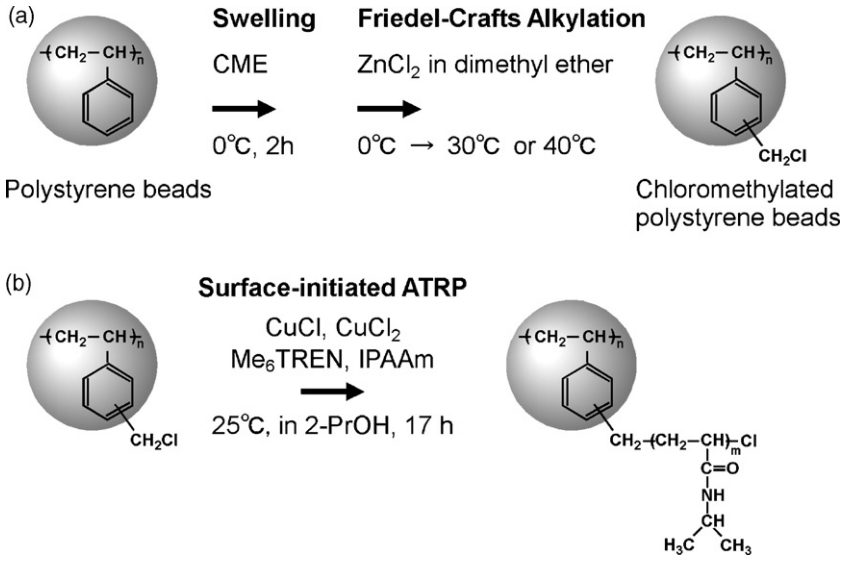

Fig. 1. Scheme for (a) the preparation of ATRP initiator-immobilized porous polystyrene beads by Friedel-Crafts reaction, and (b) the preparation of PIPAAm brush-grafted porous polystyrene beads by surface-initiated ATRP.