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Biosynthesis of superparamagnetic polymer microbeads via simple precipitation of enzymatically synthesized short-chain amylose

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ABSTRACT

Here, we report a simple and non-emulsion based approach to prepare starch-based magnetic polymer beads with well-defined size, shape and dispersibility in aqueous environment through co-precipitation of enzymatically synthesized short chain amylose (SCA) and dextran-coated iron oxide nanoparticles (Dex@IONPs). The size and morphology of magnetic polymer beads (MPBs) were controlled by employing Dex@IONPs as a seeding agent. The resulting superparamagnetic amylose microbeads (SAMBs) were readily functionalized with antibody through one step reaction using a linker protein, which showed great capture efficiency (> 90%) and specificity for target bacteria present in complicated food matrix. The excellent magnetic sensitivity also enabled the SAMBs readily assembled into ordered 1D arrays by external magnetic field whose structure could permanently be fixed by SCA-mediated precipitation process.

1. Introduction

Magnetic polymer beads (MPBs) have attracted extensive attention due to their excellent properties of instant action and contactless control driven by the external magnetic field. With combination of inherent features of magnetic particles and polymer, MPBs are used for various biological applications, such as bioseparation, drug delivery, magnetic resonance imaging (MRI), hyperthermia treatment and biosensing (Laurent et al., 2008). Among them, immunomagnetic separation is a powerful and widely used technique in bioassay through the selective concentration and isolation of the biological and chemical target molecules from a complex and heterogeneous matrices (Haukanes & Kvam, 1993). Synthesis of MPBs can be carried out by a number of methods, including emulsion polymerization (Xu, Cui, Tong, & Gu, 2006), hydrothermal reaction (Wang, Sun, Sun, & Chen, 2003), sol-gel synthesis (Lu, Yin, Mayers, & Xia, 2002), microwave irradiation (Khollam et al., 2002), sonochemical synthesis (Suslick, Fang, & Hyeon, 1996), etc. along with *in-situ* coatings or post-synthesis coatings using various polymers, such as dextran, chitosan, alginate, polystyrene, polyacrylamide, polyvinyl alcohol, and polyethylene glycol (Laurent et al., 2008; Philippova, Barabanova, Molchanov, & Khokhlov, 2011). However, these methods are still facing substantial challenges, such as potential toxicity, complicated process, and high energy consumption, which limit their applications and large-scale production.

Amylose could be a promising polymer for synthesis of MPBs due not only to its abundance, renewable nature, low cost, and biodegradability, but also to its intrinsic properties of crystallization and self-association in aqueous solutions without need of energy consumption (Roblin et al., 2012). Recently, we have reported that short chain amylose (SCA, DP ≈ 45) synthesized by amylosucrase from *Deinococcus geothermalis* (DgAS) using sucrose as a sole substrate could spontaneously self-assemble into spherical microstructure (Lim, Seo, Jung, Park, & Kim, 2014; Lim, Park et al., 2016; Potocki-Veronese et al., 2005). In addition, a guest agent, such as iron oxide nanoparticles (IONPs) were shown to be incorporated into the microstructure during the self-assembly process (Lim et al., 2015; Lim, Lee et al., 2016). However, the inefficient incorporation of IONPs inside the particles along with the undesirable aggregation of the microparticles decreased the magnetic sensitivity and effective surface area of the particles.

The self-assembly of amylose microcrystal is spontaneous precipitation process, in which the SCAs were self-associated into a polycrystalline agglomerate. Given this 'conventional' precipitation process, kinetic control over the nucleation and growth steps should be taken into an account to achieve the well-defined dimensions of amylose microstructure. One of promising approaches to control the supramolecular assembly is seeded reaction (Ma et al., 2016; Ogi, Stepanenko, Sugiyasu, Takeuchi, & Würthner, 2015; Pal et al., 2015), in which the kinetics of precipitation or self-assembly could be modulated by the addition of nuclei or seeds (Fukui et al., 2017; Ogi, Sugiyasu, Manna, Samitsu, & Takeuchi, 2014). Herein, we employed a 'seeding' approach, where dextran-coated IONPs (Dex@IONPs) were introduced as 'seeds', for fabrication of monodisperse superparamagnetic amylose

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microbeads (SAMBs) with a well-defined shape and size and a strong response to an external magnetic field through kinetic control of precipitation of SCA in aqueous solution. The SAMBs were also assembled into a 1D particle chains under external magnetic field during the self-assembly process.

2. Material and methods

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), dextran (Mw 9000–11000), γ-Fe_2O_3, Tris–HCl, lysozyme, and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sucrose and phosphate buffer saline (PBS) were obtained from Noble Bio (Suwon, Korea). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium hydroxide, and acetone were purchased from Daejung (Siheung, Korea). Ampicillin was supplied by Biosesang (Seongnam, Korea). Anti-*Escherichia coli* O157 monoclonal antibody (FITC conjugate) was purchased from Thermo Fisher Scientific Inc. (Cambridge, MA, USA). All restriction enzymes were acquired from New England Biolabs (Ipswich, MA, USA). Ni-NTA Superflow resin was obtained from Qiagen (Valencia, CA, USA).

2.2. Synthesis of dextran-coated iron oxide nanoparticles (Dex@IONPs)

Dex@IONPs were synthesized by the procedure reported by Ahmadi with modification (Ahmadi et al., 2011). Briefly, 80 mmol of $FeCl_3{\cdot}6H_2O$, 40 mmol of $FeCl_2{\cdot}4H_2O$, and 150 mg of dextran were dissolved in 20 ml deionized water (DW). The mixture was bubbled through a gentle stream of nitrogen gas for 2 min, and then ultrasonicated by Q500 Sonicator (VC 750, Sonics & Materials Inc., Newtown, CT, USA) with a 5 s disruption period at 5 s intervals in an ice bath for 3 min at 25% amplitude concurrently using a 6-mm of ultrasound probe. During the sonication, 60% ammonium hydroxide solution was added dropwise into the mixture using pipette until the mixture turned into dark suspension. The synthesized Dex@IONPs was washed several times with absolute ethanol and deionized water to remove residue of ammonium hydroxide and dextran. The final product was stored at 4 °C until use.

2.3. Characterization of Dex@IONPs

Fourier transform infrared (FT-IR) spectra for dextran, IONPs and Dex@IONPs were recorded using a Perkin-Elmer Spectrum One System spectrometer (Foster City, CA, USA) with KBr pellets in the range of 500–4000 cm^{-1}. The crystal structures of Dex@IONPs and γ-Fe_2O_3 were analyzed by powder x-ray diffraction (XRD) from 20° to 70° (2θ) using Cu Kα radiation on a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany). The morphology and size of the prepared Dex@IONPs were analyzed by transmission electron microscopy (TEM) and Dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments). All samples were dehydrated in a vacuum desiccator before the analysis.

2.4. Preparation of superparamagnetic amylose microbeads (SAMBs)

Short chain amylose (SCA) was prepared by enzymatic polymerization using amylosucrase from *Deinococcus geothermalis* (DGAS) as described earlier (Lim et al., 2014). The synthesized Dex@IONPs were mixed in a reaction solution consisting of 50 mM Tris-HCl (pH 8.0), 500 mM sucrose, and 500 units of DGAS. The total volume of reaction solution was brought up to 1 ml with DW, and incubated at 40 °C for 48 h. The final product was washed 3 times with DW and ethanol in combination with a magnetic separation method, and stored in 20% ethanol at 4 °C until needed. The morphology and composition of the synthesized SAMBs were analyzed by high-resolution scanning electron microscopy (HR-SEM) and TEM equipped with EDS elemental mapping of iron, carbon and oxygen. Magnetic properties of SAMBs and commercial polystyrene based magnetic beads, Dynabeads, with a particle diameter of 1.0 μm (Invitrogen, Carlsbad, CA, USA) were measured using physical property measurement system (16 T PPMS Dynacool, Quantum Design, USA) at room temperature from −12000 to 12000 Oe.

2.5. Preparation of SAMBs in rod form

SAMBs were transformed into one-dimensional rod-like form by exposure to external magnetic field in combination with coating the rod structure with SCA. In other words, the aligned SAMBs in rod-form by external magnetic field were further solidified by precipitation of SCA on the surface of rod structure SAMBs. Briefly, 5 mg/ml of synthesized SAMBs were suspended in a solution consisting of 50 mM Tris-HCl (pH 8.0), 500 mM sucrose, and 500 units of DGAS. The volume of reaction solution was brought up to 1 ml with DW, and incubated at 40 °C for 4 h with a magnet block (5 cm × 5 cm × 3 cm) placed at 7 cm away from the reaction tube. The prepared rod-like SAMBs was washed 3 times with DW and ethanol, respectively, and stored at 4 °C until use.

2.6. Preparation of maltose binding protein-tagged green fluorescent protein (MBP-GFP) and MBP-tagged streptococcal protein G (MBP-SPG) fusion protein

The recombinant MBP-GFP and MBP-SPG fusion protein was prepared according to the method reported previously (Lim, Lee et al., 2016). Briefly, *Escherichia coli* DH5α harboring the MBP-GFP-His expression vector and MBP-SPG-His expression vector were, respectively, cultured in 500 ml LB broth containing ampicillin (0.1 mg/ml) at 37 °C with shaking at 250 rpm. After reaching an OD_{600} of 0.7–0.8, 0.1 mM IPTG was added to induce overexpression of the fusion protein and incubated further at 18 °C for 18 h. The cells were harvested by centrifugation (4000*g* for 20 min at 4 °C) and resuspended in 5 ml of a lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, and10 mM imidazole, pH 8.0) for 20 min at 4 °C, followed by sonication for 10 min with a 10 s disruption period at 10 s intervals in an ice bath at 20% AMP. After centrifugation at 4000*g* for 20 min, the supernatant was passed through a column packed with Ni-NTA resin. The Ni-NTA column was washed with a washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0), and the MBP-SPG proteins were eluted with an elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). The purified MBP-SPG was stored at 4 °C until needed.

2.7. Preparation of immuno-SAMBs: functionalization of SAMBs with MBP-SPG fusion protein

To immobilize the antibodies to the surface of the SAMBs the recombinant MBP-SPG fusion protein was used as a cross-linker with the specific affinity of MBP and SPG to amylose and the Fc region of the antibody, respectively. The prepared SAMBs were suspended in a reaction solution containing 30 μg/ml of MBP-SPG, incubated at 4 °C for 60 min in a rotary shaker, washed 3 times with PBS (pH 7.4), and then resuspended in PBS to a final concentration of 50 mg/ml. The anti-*E. coli* O157 antibody or FITC-labeled anti-*E. coli* O157 antibody were mixed with the MBP-SPG-SAMBs to a final concentration of 2 μg/ml. After incubating at 4 °C for 90 min in a rotary shaker, antibody-functionalized SAMBs were washed 3 times with PBS and stored at 4 °C until needed. The conjugation of FITC-labeled antibodies on the surface of the SAMBs were confirmed by fluorescence microscopy (Nikon TE2000U, Tokyo, Japan).

2.8. Immunomagnetic separation

Freshly cultured *E. coli* O157:H7 was diluted serially to the