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Enzyme-polymer hybrid nanogels fabricated by thiol-disulfide exchange reaction

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ABSTRACT

In this paper a novel method for the fabrication of hybrid nanogels based on thiol-disulfide exchange reaction is reported. Poly(oligo(ethylene glycol) monomethyl ether methacrylate-*co*-di(ethylene glycol) methyl ether methacrylate-*co*-2-(2-pyridyldisulfide) ethyl methacrylate) (POEGMA-*co*-PDEGMA-*co*-PDSMA) was synthesized by reversible addition-fragmentation chain transfer polymerization. Pyridyl disulfide functionalized porcine pancreatic lipase (PPL-S-S-Py) was prepared by treatment of PPL with Traut's reagent (2-iminothiolane) and 2,2′-dithiodipyridine. Upon addition of meso-2,3-dimercaptosuccinic acid into aqueous solutions of PPL-S-S-Py and POEGMA-*co*-PDEGMA-*co*-PDSMA, enzyme-polymer hybrid nanogels were prepared. The hybrid nanogels show thermal responsiveness. With an increase in the content of PPL in the nanogels, the lower critical solution temperature (LCST) shifts to the higher temperature. At a temperature below LCST, PPL molecules are in the shells of the nanogels, and at a temperature above LCST, PPL molecules are embedded inside the nanosized structures. The immobilized PPL show enhanced heat resistance and good reusability.

1. Introduction

Enzymes as remarkable biocatalysts are capable of catalyzing a wide array of complex reactions with exquisite selectivity [1]. The enzyme molecules have delicate three-dimensional structures, but they suffer from poor stability including chemical degradation as well as physical unfolding and aggregation [2]. In practical applications enzymes are usually immobilized on different carriers to improve their properties, including reusability, operational stability, and recovery [3]. Immobilized enzymes have many advantages over free enzymes, such as enhanced stability, improved activity, and ease of separation from the reaction systems [4]. The immobilization methods can be catalogued into coupling immobilization (physical adsorption or covalent bonding) and encapsulation immobilization [5]. The physical adsorption based on electrostatic or hydrophobic interaction is a simple method to immobilize enzymes, however, the immobilized enzymes are easily released from the carrier matrix resulting in poor stability and low activity. The covalent coupling method is able to solve the desorption problem, but this method often leads to the change of the enzyme structures and reduction of enzyme activity [6,7]. Encapsulation or entrapment of enzymes in crosslinked network structures provides an efficient method to immobilize the enzymes [8,9]. For example, Chen and coworker synthesized core-shell particles composed of styrene, *N*-isopropylacrylamide (NIPAAm), and *N*-acryloxysuccinimide (NAS) by surfactant-free emulsion polymerization. The latex particles were used for immobilization of α-chymotrypsin through covalent bonding with the reactive ester groups of NAS [10]. They also immobilized r-amylase in the hydrogel membrane by the formation of covalent bonds between the enzyme and the ester groups in NAS [11]. Hamerska-Dudra and coworkers synthesized PNIPAAm gels with hydroxyl, epoxy, or amino groups, and immobilized glucoamylase and trypsin on the gels by means of glutaraldehyde [12].

Lipases are able to catalyze the hydrolysis of long-chain acylglycerols to glycerol, free fatty acids, and mono- and diacylglycerols, and have found applications in chemical and pharmaceutical industries [13]. Lipases usually functionalize at the interface of two-phase systems [14,15]. Porcine pancreatic lipase (PPL) is a small globular protein composed of a single chain of 449 amino acids, with a molecular weight of 50–52 kDa. In previous researches, immobilizations of PPL on different solid surfaces have been reported [16–18].

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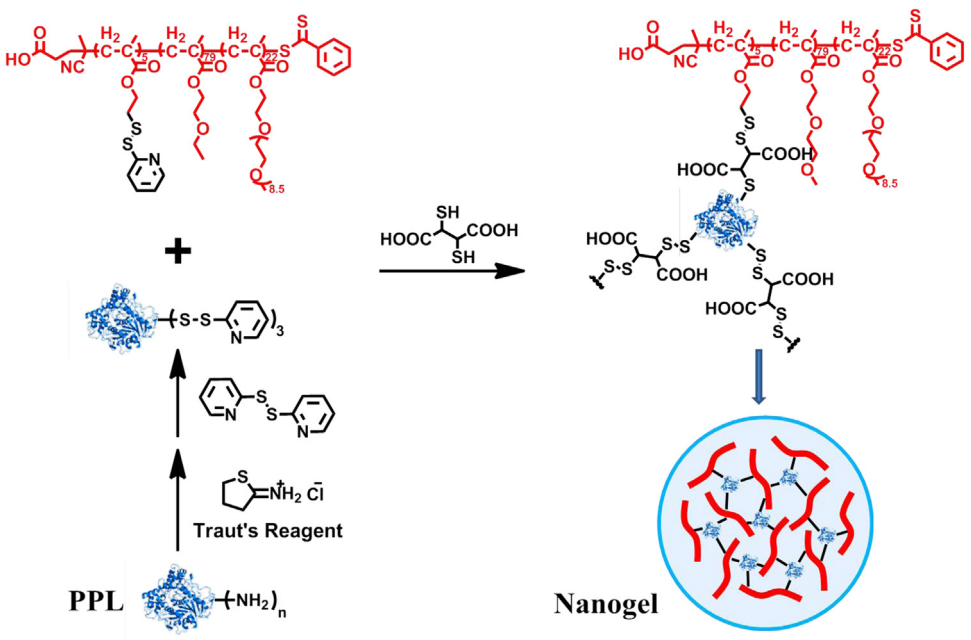

Scheme 1. Scheme for the synthesis of enzyme-polymer hybrid nanogel by thiol-disulfide exchange reaction.

Nanosized hydrogels combine the properties of both hydrogels and nanoparticles [19,20]. The nanogels with stimuli-responsive characteristics, including changes in volume, mechanical and optical properties in response to environmental variations, have been synthesized [19]. In order to improve the stability of enzyme molecules, polymer-enzyme hybrid nanogels were synthesized in this research. Poly(oligo(ethylene glycol) monomethyl ether methacrylate-*co*-di(ethylene glycol) methyl ether methacrylate-*co*-2-(2-pyridyldisulfide) ethyl methacrylate) (POEGMA-*co*-PDEGMA-*co*-PDSMA) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. Thiol-modified PPL was obtained after treatment of PPL with Traut's reagent (2-iminothiolane), a cyclic thioimidate compound for sulfhydryl addition [21–25]. After treatment of PPL with Traut's reagent, thiol groups were produced on PPL. The thiol-modified PPL (PPL-SH) was subsequently reacted with 2,2-dithiodipyridine, and pyridyl disulfide functionalized PPL (PPL-S-S-Py) was prepared. Upon addition of meso-2,3-dimercaptosuccinic acid into aqueous solutions of PPL-S-S-Py and POEGMA-*co*-PDEGMA-*co*-PDSMA, enzyme-polymer hybrid nanogels were prepared. The synthesis of the hybrid nanogel is schemed in Scheme 1. The reason why we did not prepare the hybrid nanogels by directly mixing PPL-SH and the polymer is that the thiol groups on PPL-SH are easily oxidized into disulfides in the purification and the storage process.

2. Experimental section

2.1. Materials

2-Mercaptoethanol (Tianjin Chemical Reagent Co., AR) was distilled under reduced pressure. Triethylamine (Tianjin Chemical Reagent Co., AR) was dried with $LiAlH_4$ and distilled before use. Oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA, Mn = 500 Da, 99%,) and di(ethyleneglycol) methyl ether methacrylate (DEGMA) were purchased from Sigma Aldrich and purified by passing through a column filled with neutral alumina to remove inhibitor. Chain transfer agent (CTA) (4-cyanopentanoic acid) dithiobenzoate (CPADB) was prepared by using a method similar to previous literature [26,27]. 4,4′-Azobis(4-cyanopentanoic acid) (ABCPA, Aldrich, 97%) was purified by recrystallizing in methanol and drying under reduced pressure at room temperature. 2,2′-Dipyridyl disulfide (Heowns Co., 97%), methacryloyl chloride (Heowns Co., 98%), meso-2,3-dimercaptosuccinic acid (Alfa, 97%), 2-iminothiolane hydrochloride (Sigma-Aldrich, >98%), Lipase from porcine pancreas Type II, 100–500 units/mg protein using olive oil and 30–90 units/mg protein using triacetin (Sigma-Aldrich), Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid), DTNB, Sigma Aldrich), 4-nitrophenyl palmitate (Heowns Co., 98%), were used as received. All the solvents were distilled before use.

2.2. Synthesis of 2-(pyridine-2-yldisulfanyl)ethanol

2,2′-Dipyridyl disulfide (11.8 g, 53.8 mmol) was dissolved in 100 mL of methanol, and mercaptoethanol (2.80 g, 35.9 mmol) in 30 mL of methanol was added into the solution at room temperature. After stirring overnight, the solvent was removed at reduced pressure and crude 2-(pyridine-2-yldisulfanyl)ethanol was obtained. The crude product was purified by column chromatography using a mixture of ethyl acetate and petroleumether as eluent. The yield is about 67%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS, ppm): 8.54 (d, J = 4.3 Hz, 1H), 7.68–7.55 (m, 1H), 7.41 (t, J = 12.2 Hz, 1H), 7.22–7.13 (m, 1H), 3.85–3.75 (m, 2H), 3.03–2.92 (m, 2H). ^{1}H NMR spectrum is shown in Fig. S1.

2.3. Synthesis of 2-(pyridine-2-yldisulfanyl) ethyl methacrylate (PDSMA)

A scheme for the synthesis of PDSMA is shown in Fig. S2. 2-(Pyridine-2-yldisulfanyl)ethanol (4.50 g, 24.1 mmol) in 40 mL of dry dichloromethane was mixed with triethylamine (3.60 g, 36.1 mmol) and the solution was cooled in an ice-bath. Methacryloyl chloride (3.75 g, 36.1 mmol) in 20 mL of dichloromethane was added into the solution and the solution was stirred at room temperature for 12 h. After the reaction, the solution was washed with 30 mL of distilled water for three times and with 30 mL of saturated NaCl solution, and dried over Na_2SO_4. The organic layer was concentrated on a rotation evaporator and crude yellowish 2-(pyridine-2-yldisulfanyl) ethyl methacrylate was obtained. The monomer was purified by column chromatography. The yield is about 87%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS, ppm): 8.39 (d, J = 4.5 Hz,