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Switch on/off of cellulase activity based on synergetic polymer pair system

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ABSTRACT

Cellulase has enormous potential applications in various industries, including food, textiles, and paper, etc. However, undesirable heat-induced misfolding is considered as one of the major problem in cellulase application. So, we have designed a practical and efficient synergetic polymer pair system (SPPS), which can regulate and preserve the enzymatic activity of cellulase with a pair of oppositely charged polymers. First, we designed and synthesized a copolymer, poly(ethylene- glycol)-graft-poly(N,N-dimethylaminoethyl methacrylate) (PEG-g-PDMAEMA) with hydrophilic and cationic chains, which was connected with anionic cellulase to form a water-soluble cellulase/PEG-g-PDMAEMA complex to inhibit the enzymatic activity of cellulase completely without loss of secondary structure. In the second step, the enzymatic activity of enzyme/copolymer complex was recovered successfully with the addition of an anionic polymer, poly(acrylic acid) (PAAc). What surprised us was that 81% of the enzymatic activity of the cellulase/PEG-g-PDMAEMA complex was restored after 10 min of heating at 90 °C. Circular dichroism (CD) spectral analysis clearly indicated that there was no significant impact on the conformation of the cellulase of heat-treated enzyme/copolymer complex.

1. Introduction

As a kind of biological protein catalysts, enzymes have particular catalytic characteristics, such as high efficiency and specificity. With the development of science and technology, enzymes have been deeply attracted among numerous researchers and widely used in many fields including medicinal chemistry and biotechnology. However, enzymes are prone to denaturation or inactivation with the drastic changes of their environment, such as high temperatures, high or low pHs, and organic solvents, which have become a serious obstacle to versatile applications. So, the current core work is to avoid the inactivation of enzymes and then expand the applications not only in research fields but also in industries. Based on previous studies, several methods have been developed, involving artificial chaperones [1–3], enzyme immobilization [4–6], protein engineering [7] and enzyme modifications [8–10]. Compared with other techniques, the process of enzyme modifications is simpler and the cost is quite lower [11–13].

Several researchers have designed a switching mechanism to regulate enzyme activity [14–19]. On/off switching of enzyme activity successfully solves the limitation of applications by denaturation or inactivation, which broadens the research fields including molecular diagnostics, affinity separations, bioelectronics, and biosensor technologies. One artificial system developed to achieve the on/off switching is that the covalent modifications of a ligand [20] and stimuli-responsive polymers [21–23], which is around the substrate and bind the active sites of enzyme to control enzyme activity. In some special cases, the enzyme activity can also be regulated even if the modified sites are far from the active sites [24]. This on/off switching on the regulation of enzyme activity is reversible with the stimulating signals. In addition, noncovalent interactions also play a very important role in the manipulation of enzyme activity. The function was realized by artificial inhibitors, such as polymeric substrate analogues [25], molecular tweezers and clips [26], dendrimers [27], gold nanoparticles [28–31], and micelles [32–34]. Electrostatic interactions have been generated considerable interest among many researchers, because it is more economic and more efficient compared with others, involving the hydrogen bonds, van der Waals force and hydrophobic interactions [14,16,18,35].

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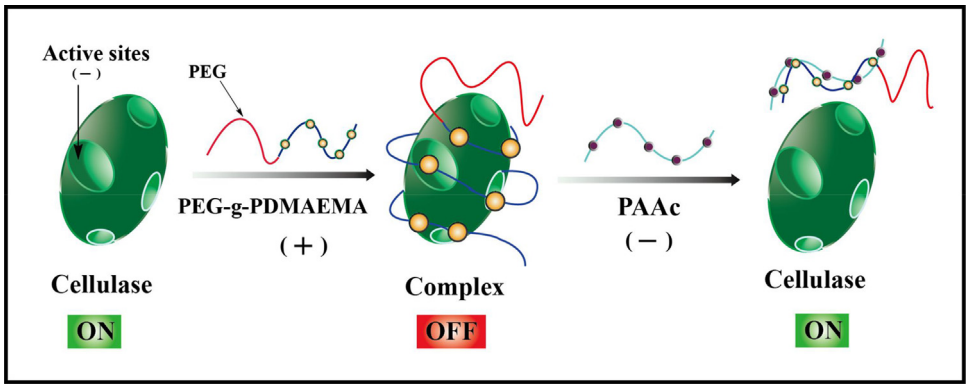
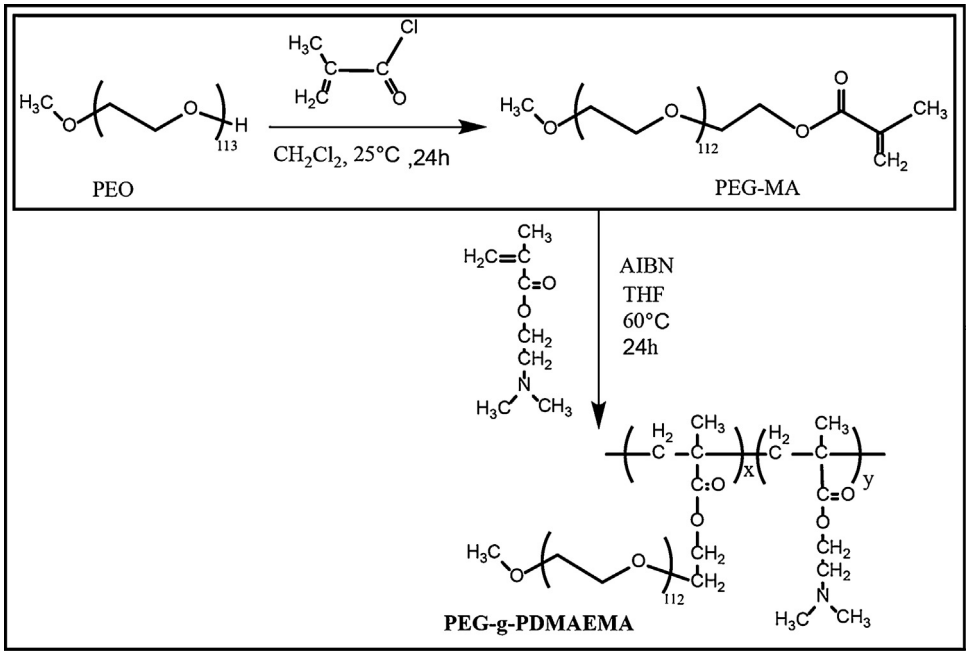


Fig. 1. Schematic illustration of synergetic polymer pair system (SPPS).



Scheme 1. The synthesis and design strategy of PEG-g-PDMAEMA.

Cellulose is the most abundant renewable resource on the earth, but without effective utilization, and cellulases have enormous biotechnological potential prospects in various industries, including textile, papermaking, food fermentation, and wastewater treatment, etc. Here, we attempt to regulate the enzyme activity of cellulase with a simple and effective system. Based on the former research of preservation of enzyme from inactivation through intermolecular electrostatic interactions, we have recently designed a convenient and effective system, synergetic polymer pair system (SPPS), which consists of a pair of oppositely charged polymers, PEG-g-PDMAEMA and PAAc to achieve on/off switching of enzyme activity of cellulase [36–39]. The cationic copolymer, PEG-g-PDMAEMA, interacted with the cellulase by electrostatic force, and therefore the active sites were covered and insulated from the external environment, which effectively prevented the cellulase from aggregation and resulted in complete inhibition of the enzyme activity of cellulase. The PEG chains are hydrophilic and nontoxic, which provide a safe space for the cellulase and increase the solubility of the copolymer and also promote the stability of the enzyme/copolymer complex [37]. The enzyme activity of cellulase/PEG-g-PDMAEMA complex was recovered with the addition of an anionic polymer, PAAc (Fig. 1). The recovery of the enzyme activity of cellulase/PEG-g-PDMAEMA complex was quite effective, although the cellulase/PEG-g-PDMAEMA complex was heated at high temperature. Circular dichroism (CD) spectral analysis indicated that there was no remarkable conformational change of the cellulase of the heated cellulase/PEG-g-PDMAEMA complex. These facts suggested that the electrostatic interaction between cellulase and PEG-g-PDMAEMA prevented the cellulase from denaturation under hyperthermia [36,37]. However, the specific reaction principle of copolymer with cellulase is not clear.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) monomethyl ether (MeO-PEG-OH, M_n = 5000 g/mol) purchased from Aldrich (Shanghai, China) was used as received. CH_2Cl_2 obtained from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China) was purified through vacuum filtration after being dried over CaH_2. Methacryloyl chloride and tetrahydrofuran (THF) were from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China) without further purification. 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Aldrich (Shanghai, China) and was recrystallized from 95% ethanol. 2-