Biochemical Engineering Journal 34 (2007) 217–223

Biochemical Engineering Journal

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Neutral lipase from aqueous solutions on chitosan nano-particles

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Received 21 December 2005; received in revised form 21 September 2006; accepted 7 December 2006

Abstract

The size of chitosan nano-particles had been prepared by ionic cross-linking of chitosan and tri-polyphosphate (CS-TPP), which were well dispersed and stable in aqueous solution. The physicochemical properties of nano-particles were characterized by IR spectra, SEM. Its sorption kinetics and sorption mechanism for neutral lipase were studied. The effect factors of adsorption kinetics were investigated, such as neutral lipase concentration, chitosan nano-particles solution concentration, adsorption temperature, size of chitosan nano-particles, stirring rate, solution pH, etc. Adsorption of neutral lipase on chitosan nano-particles was fitted into Lagergren first-order equation at initial neutral lipase of 3.0 mg/ml (pH 7.0). The first-order constant for neutral lipase was 23.34 h^{-1}. When neutral lipase concentration was controlled under certain region, it was fitted into Freundlich isothermal linear equation. Mechanism of adsorption for neutral lipase was presumed by analyzing IR spectra. The hydrogen in the carboxyl group was connected with electronegative oxygen. Electron pair was attractive to oxygen, so hydrogen atom became cation and proton. When electronegative neutral lipase was closed to chitosan nano-particle, the hydrogen bond might be formed. It was the main force between hydroxyl group, NH_2 group and neutral lipase.

Keywords: Chitosan; Nano-particles; Neutral lipase; Sorption kinetics; Sorption mechanism

1. Introduction

Biopolymers, chitosan is the deacetylated form of chitin and composed of glucosamine, known as (1–4)-2-amino-2-deoxy-D-glucose. Chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively [1]. This special structure makes it exhibit chelation with various metal ions [2]. Muzzarelli [3] pointed out that chitosan combines with metal ions by three forms: ion exchange, sorption and chelation. Chitosan has been broadly used for the sorption of heavy metal ions [4–6]. Further physical and chemical modifications of chitosan have been made to improve the selectivities and the capacities for metals ions [7–10]. Chitosan is also characterized by weak diffusion properties: long contact times are required to reach equilibrium. Sorption capacity can be controlled by sorbent particle size [8]. Due to the low porosity of chitosan, sorption performances are frequently controlled by mass transfer resistance. To reduce this resistance to mass transfer, chitosan gel beads have been developed to expand the polymer structure and reduce its crystallinity [11–13]. However, these treatments results in either a decrease of the number of available sorption sites (cross-linking treatment), or the volumetric sorption capacity (the percentage of water in the gel beads can reach 95%) [14]. Controlled drying can increase the volumetric sorption capacity [15]. Another possibility for increasing this volumetric sorption capacity is the grafting of supplementary functional groups [16].

A number of nano-scale inorganic particles offer favourable properties in regard to selective removal of target contaminants. For example, hydrated Fe(III) oxides particles can selectively adsorb dissolved heavy metals like zinc, copper or metalloids

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doi:10.1016/j.bej.2006.12.022

like arsenic oxyacids or oxyanions [17]. Amorphous and crystalline Fe(III) hydroxides have long been known to possess selective sorption properties toward arsenites and arsenates [18]. Polymer supported nano-particles have been prepared and used for selective removal of target arsenic compounds and heavy metals [17]. Chitosan nano-particles had been synthesized based on polymer and applied as drug carriers as reported in previous studies [19–21].

At present, most of chitosan carriers used as immobilized lipase enzyme were micro-sphere, film, micro-capsule, power, etc. [22–25]. Researches about the sorption properties of chitosan nano-particles for enzyme/protein are rarely reported now. The unique character of nano-particles for their small size, great surface area and quantum size effect could make it exhibit higher capacities for neutral lipase. Chitosan nano-particles used as immobilized enzyme carriers had been studied by our group. The results about immobilized enzyme characters and comparison with other chitosan carriers had been published [26]. The work in this paper aimed to study its sorption kinetics and sorption mechanism for neutral lipase.

2. Materials and methods

2.1. Materials

Neutral lipase (5000 U/g) was a commercial enzyme, of food-grade, obtained from Wuxi Enzyme Reagent Factory (Jiangshu, China). Chitosan was provided by Yuhuan Ocean Biochemical Co. Lit (Zhejiang, China), the molecule weight of which was 91,000. TPP abbreviated from sodium polyphosphate was purchased from Dongsheng Chemical Reagent Factory (Zhejiang, China). All other chemicals were of analytical grade and no further purification was required.

2.2. Preparation of chitosan nano-particles

Twenty milligram of chitosan was dissolved in 40 ml of 2.0% (v/v) acetic acid. Twenty millilitres of 0.75 mg/ml TPP was dropped into the beaker. Then the solution was treated with super-filtration membrane to remove the residual TPP. Chitosan nano-particles were stored up in the distilled water.

2.3. Morphology and structure characterization of chitosan nano-particles

Chitosan nano-particles were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Coated samples were examined using a XL30-SEM scanning electron microscope to characterize the morphology and size of nano-particles.

FT-IR of chitosan nano-particles were taken with KBr pellets on Nicolet Nexus 670 Spectrum FT-IR. The samples were chitosan, chitosan nano-particles, chitosan nano-particles adsorbed neutral lipase, respectively.

2.4. Sorption kinetics experiments

Batch experiments for determination of kinetics of neutral lipase on chitosan nano-particles were carried out using a continuously stirred glass vessel. Different concentrations of different mean particle sizes chitosan nano-particles solutions were brought in contact with 1.0 ml of neutral lipase solutions under continuous stirring, at temperature (30–60 °C). The pH was 7.0. The initial concentration of neutral lipase was varied to investigate their effect on the sorption kinetics. During the kinetic experiments, samples were withdrawn at fixed time intervals, filtered, and analyzed with an UV/vis spectrometer 751 (Shanghai, China).

2.5. Equilibrium experiments

Batch equilibrium experiments were carried out using chitosan nano-particles as sorbent. A series of flasks containing neutral lipase solutions of varying concentrations prepared from neutral lipase and a fixed concentration of chitosan nano-particles were agitated in a rotary shaker at 200 rpm at room temperature. Neutral lipase uptake experiments were conducted under pH value 7.0. After equilibration, neutral lipase solutions were filtered through papers and analyzed. Neutral lipase-free and sorbent-free blanks were used as controls. Amounts of neutral lipase taken up by the sorbent in each flask were determined by the following mass balance equation:

$$Q = \frac{V(C_0 - C_e)}{W}$$

where Q is the sorption capacity (mg/g), C_0 and C_e are, respectively, the initial and solution phase neutral lipase concentration at equilibrium (mg/l), V the solution volume (l), and W is the mass of sorbent (g).

3. Results and discussion

3.1. Characterization of chitosan nano-particles

3.1.1. Size and morphology of chitosan nano-particles

The preparation of chitosan nano-particles was based on an ionic gelation interaction between positively charged chitosan and negatively charged tri-polyphosphate at room temperature [28,29]. Chitosan nano-particles prepared in the experiment exhibited white powder shape, and were insoluble in water, dilute acid, and alkalescent solution. Results were followed in Fig. 1.

3.1.2. Surface functional groups of chitosan nano-particles

The sorption capacity of chitosan could be improved by the substitution of various functional groups, such as amino acid esters, oxo-2-glutaric acid and pyridyl [30–32]. Several phosphorus derivatives of chitosan have also been synthesized by grafting phosphate or phosphonic acid [33–35]. Tri-polyphosphate has also been selected as a possible cross-linking agent for the preparation of chitosan gel beads by the coagulation/neutralization effect. Spherical chitosan tripolyphosphate chelating beads had been prepared and applied