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Concanavalin A induced orientation immobilization of Nuclease P_1: The effect of lectin agglutination

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ABSTRACT

Orientation immobilization of enzymes has attracted intensive interest owing to the retainable specific activity and stability. Specially, glycoprotein immobilized onto Concanavalin A (Con A) modified carriers induces the orientation of the enzyme. However, the effects of the interface properties of carriers and enzymes are still not well understood yet. In this study, we synthesized the activated porous poly (styrene- divinylbenzene) resin carriers with 30 nm pore sizes and 72 $m^2 g^{-1}$ specific surface areas and decorated with Con A. The resultant loading capacity of NP_1 on Con A modified carriers was as high as 4.02 mg g^{-1} wet support as a result of strong affinity between the enzyme and Con A decorated on carriers. It was found that the acid resistance, thermal stability, reusability and degradation efficiency of the immobilized enzyme on Con A modified porous carriers were significantly improved. The reduction of K_m from 18.40 ± 0.55 mg mL^{-1} to 17.19 ± 0.51 mg mL^{-1} illustrated the improved substrate affinity of HA-GA-ConA-NP_1. Moreover, Con A-affinity NP_1 exhibited the best operational stability that only 7% of its initial activity was lost even after 9 batches repeated reaction. This work demonstrates that surface property manipulation of porous carriers and its derivatives has great potential in efficient biocatalytic systems.

1. Introduction

Nowadays, enzyme catalysts have attracted ever increasing attention in industrial applications due to the advantages of substrate specificity and green nature of the reactions. Immobilizing enzyme onto substrate is favored in terms of recyclability and stability when compared to soluble enzyme [1–3].

Nuclease (Nucleic acid enzyme) P_1 (NP_1, EC 3. 1. 30. 1) firstly recognized by Kuninaka et al. from Penicillium citrinum is a glucoproteinase with 17.4% glycosyl, such as mannose, glucose and galactose [4,5]. NP_1 has been used not only for industrial hydrolysis of macromolecular substance RNA and heat-denatured DNA to produce 5′- nucleotides (AMP, GMP, CMP and UMP) from the 3′- hydroxy termini, but also for analysis of the 5′- terminal nucleotide sequences of several viral RNA's. The products of the NP_1 catalysis, 5′- ncleotides, have been widely used in the area of food, medicine, agriculture etc [4,6–8]. However, the instability in high temperature restricts the industrial application of NP_1.

The previous studies in our laboratory and others have somehow solved this problem by successful immobilization of NP_1 on natural and synthetic supports, such as ion exchange resins [9], cellulose [10], macroporous absorbent resin [11], weak base anion resin [12], PEG-NH_2 modified graphene oxide [7] based on either physical adsorption or covalent crosslinking. Physical adsorption is a temperate method and can achieve high activity because of its little negative effect on the conformation of enzyme. But the enzyme may release into the solution used for its slight interaction and go against the reusability.

Although covalent protocol is more stable, the direct interaction with some essential amino acid residue in the strategy may hinder active sites, diminish its catalytic efficiency and restrict the applications of immobilized enzyme [1,4–8]. Functional chemical group may promote mobility of the biocatalyst allowing a better dynamic motion for interaction of the protein with a substrate or an optimized molecular orientation of enzymes attached to surfaces [13,14]. Perhaps, oriented-

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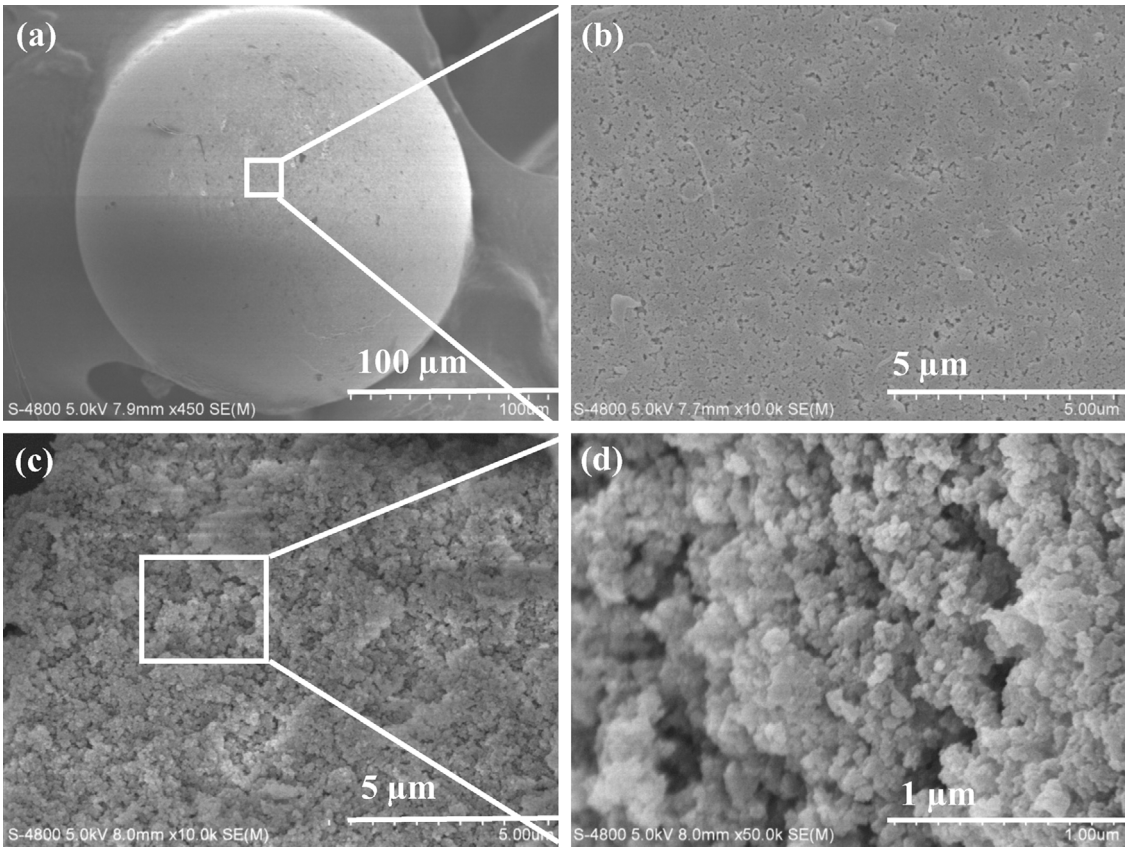

Fig. 1. Field emmision scanning electron microscopy (FESEM) images of activated amino resin porous carriers: (a) the surface of resin particle, (b) the magnification of surface structure as shown in (a), (c) the internal structure of the resin particle, and (d) the magnification of inner structure as shown in (c).

immobilized strategy on tailor-made supports with functional chemical group is promising and interesting that can address the issue [15,16]. Nevertheless directed immobilization of NP_1 via strong electrostatic and hydrophobic interactions between the enzyme and the carrier can somewhat increase the enzyme activity [7,17], but the process is difficult to control and the affinity to substrate has not been improved yet.

Con A, extracted from jack bean, is one of the most studied lectin. It is a metal protein including 237 different amino acids, and can specifically recognize mannose and glucose to form a sandwich structure via strong bio-affinity interaction in soft manner [18–21]. At neutral condition, Con A is a tetramer including a glycosyl binding site with two metal sites in each subunit [21]. The metals may directly stabilize the saccharide-binding residues in their active conformation and enhance the stability of glycoproteins [17,22].

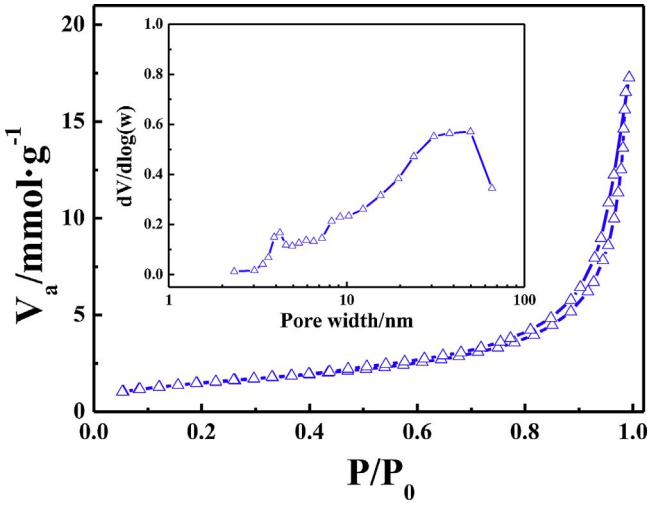

Fig. 2. Textural analysis of activated amino resin porous carriers. N_2 adsorption-desorption isotherm and pore size distribution curve (inset).

Compared with conventional directed-immobilization via covalent binding, the target groups used as binding sites for affinity-immobilization is far away from the enzyme's active site and thus avoids the space steric hindrance and facilitates the expression of enzyme and reusability [20,23,24]. Affinity interaction based on lectin-carbohydrate has been widely accepted in affinity-controlled glucoproteinase immobilization [25]. Con A-immobilization strategy has been successfully used in affinity purification, immobilization and separation of S1 Nuclease, glucose oxidase (GOx) [17,26], horseradish peroxidase (HRP) [27], β-Galactosidase [28], laccase [29], and catalase [30] etc. Oriented-immobilized strategy on account of (bio)affinity bonds between an pre-activated support with Con A and a specific group of the protein can be one of the most effective remedy to achieve higher enzyme activity recovery and better stability [28,29,31].

Given S_1 nuclease from Aspergitlus oryzae bounded through its carbohydrate moiety to Con A-Sepharose showed marginal increase in K_m and enhanced stability toward 8 M urea [32]. A new oriented immobilization mechanism of NP_1 based on the specific affinity links between glycosyl and Con A was proposed to enhance its stability and activity. Firstly, Con A was covalently linked on the pre-activated amino resin microspheres. Then NP_1 was immobilized on Con A-modified microspheres based on the bioaffinity interaction between carbohydrate of enzyme and Con A. FESEM, XPS, TGA and FT-IR characterization were utilized to verify the binding efficiency of Con A on porous carriers. The introduction of Con A on porous carriers offers additional possibility in nucleotide industrial biocatalytic production systems.