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Nanostructures for enzyme stabilization

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Abstract

Recent breakthroughs in nanotechnology have made various nanostructured materials more affordable for a broader range of applications. Although we are still at the beginning of exploring the use of these materials for biocatalysis, various nanostructures have been examined as hosts for enzyme immobilization via approaches including enzyme adsorption, covalent attachment, enzyme encapsulation, and sophisticated combinations of methods. This review discusses the stabilization mechanisms behind these diverse approaches; such as confinement, pore size and volume, charge interaction, hydrophobic interaction, and multipoint attachment. In particular, we will review recently reported approaches to improve the enzyme stability in various nanostructures such as nanoparticles, nanofibers, mesoporous materials, and single enzyme nanoparticles (SENs). In the form of SENs, each enzyme molecule is surrounded with a nanometer scale network, resulting in stabilization of enzyme activity without any serious limitation for the substrate transfer from solution to the active site. SENs can be further immobilized into mesoporous silica with a large surface area, providing a hierarchical approach for stable, immobilized enzyme systems for various applications, such as bioconversion, bioremediation, and biosensors.

Keywords: Enzyme stabilization; Nanostructures; Enzyme adsorption; Covalent attachment; Nanoparticles; Nanofibers; Mesoporous silica; Sol–gel; Enzyme encapsulation; Single enzyme nanoparticles

1. Introduction

Enzymes are ubiquitous natural biocatalysts of nanometer scale. The potential applications of enzymes are well recognized (Ball, 2001; Koeller and Wong, 2001; Schmid et al., 2001). Practical use of enzymes has been realized in various industrial processes and products including laundry detergents, and is being expanded in new fields: fine-chemical synthesis, pharmaceuticals, biosensors, bioremediation, biobleaching, polymerase chain reaction, protein digestion in proteomic analysis, and biofuel cells. The specificities of enzyme catalysts promise improvements in many applications, but the short lifetimes of enzymes presently limit their usefulness. Improvements in enzyme stability can enable further practical applications. It can reduce the required amount of enzymes, prolong the lifetime of enzyme reactors, increase the potential for enzyme reuse, or maintain the good signal of biosensors.

There have been many approaches to improve the enzyme stability: enzyme immobilization, enzyme modification, protein engineering, and medium engineering. Enzyme immobilization represents the attachment or incorporation of enzyme molecules onto or into large structures, via simple adsorption, covalent attachment, or encapsulation (Tischer and Kasche, 1999; Livage et al., 2001). Especially, the multipoint attachment between enzyme molecules and host materials reduces protein unfolding, and hence improves stability (Mozhaev et al., 1990). Recent developments with cross-linked enzyme crystals (CLECs) and cross-linked enzyme aggregates (CLEAs) are based on multipoint attachment between enzyme crystals (or molecules) (Haring and Schreier, 1999; Cao et al., 2000; Lopez-Serrano et al., 2002; O'fagain, 2003). Enzyme modification is defined by the covalent reactions to the enzyme molecule. Addition of

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functional groups or polymers on the surface of enzyme molecules may be used to change the surface properties, and result in an improvement of enzyme stability (Mozhaev et al., 1990; Mozhaev, 1993, Desantis and Jones, 1999; Govardhan, 1999). Protein engineering involves changing the amino acid sequence of an enzyme in order to yield an intrinsically more stable structure using molecular biology techniques such as directed evolution or site-specific mutagenesis (Arnold et al., 2001; Lehmann and Wyss, 2001; Brannigan and Wilkinson, 2002; O'fagain, 2003). Reaction medium engineering, on the other hand, is to make the enzyme structure more stable by changing the medium around it rather than the enzyme structure. This approach can be achieved either by the use of nonaqueous organic solvents or by changing the salt composition of an aqueous solution (Mozhaev, 1993; Klibanov, 2001; Lee and Dordick, 2002).

The present review is not intended to describe all the details of enzyme stabilization methods, but rather describes recent developments in the field of nanotechnology for enzyme stabilization. Various nanostructures, generally providing a large surface area for the immobilization of enzyme molecules, have been actively developed for enzyme stabilization. This review will discuss the role of those nanostructures in stabilizing the enzymes. In more detail, we will discuss the enzyme stabilization using nanoparticles, nanofibers, mesoporous silica and nanoparticles prepared via sol–gel encapsulation. In addition, we recently developed a new enzyme composite of nanometer scale that we call "single-enzyme nanoparticles (SENs)" (Kim and Grate, 2003). In a form of SENs, each enzyme molecule is surrounded with a porous composite organic/inorganic network of less than a few nanometers thick. This approach represents a new type of enzyme-containing nanostructure. We will describe the synthesis and stability of SENs, and preliminary results from the immobilization of SENs in mesoporous silica.

2. Nanoparticles

Enzyme immobilization has been a popular strategy for most large-scale applications due to the ease in catalyst recycling, continuous operation, and product purification. Poor biocatalytic efficiency of immobilized enzymes, however, often limits the development of large-scale bioprocessing to compete with traditional chemical processes (Caruana, 1997; Demirjian et al., 1999). Improvements of biocatalytic efficiency can be achieved by manipulating the structure of carrier materials for enzyme immobilization. Nonporous materials, to which enzymes are attached to the surfaces, are subject to minimum diffusion limitation while enzyme loading per unit mass of support is usually low. On the other hand, porous materials can afford high enzyme loading, but suffer a much greater diffusional limitation of substrate. For example, the value of effectiveness factor (η) for α-chymotrypsin (CT) was reported to be ≈ 0.3 when it is entrapped in polyacrylamide hydrogel (Martinek et al., 1977); below 0.1 when incorporated into hydrophobic plastics (Wang et al., 1997); and less than 10^{-3} when cross-linked (Cerovsky and Jakubke, 1994).

Reduction in the size of enzyme-carrier materials can generally improve the efficiency of immobilized enzymes. In the case of surface attachment, smaller particles can provide a larger surface area for the attachment of enzymes, leading to higher enzyme loading per unit mass of particles (Jia et al., 2003). In the case of enzyme immobilization into porous materials, much reduced mass-transfer resistance is expected for smaller porous particles owing to the shortened diffusional path of substrates when compared to large-sized porous materials. There have been extensive studies on the use of micrometer-sized particles for the enzyme immobilization (Xu and Klibanov, 1996; Govardhan, 1999; Haring and Schreier, 1999). Recently, a growing interest has been shown in using nanoparticles as carriers for enzyme immobilization (Daubresse et al., 1996; Martins et al., 1996; Caruso and Schuler, 2000, Chen and Su, 2001; Liao and Chen, 2001; Jia et al., 2003). The effective enzyme loading on nanoparticles could be achieved up to 10% wt due to a large surface area per unit mass of nanoparticles (Chen and Su, 2001). Overall, nanoparticles provide an ideal remedy to the usually contradictory issues encountered in the optimization of immobilized enzymes: minimum diffusional limitation, maximum surface area per unit mass, and high enzyme loading.

In addition to the promising performance features, the unique solution behaviors of the nanoparticles also point to an interesting transitional region between heterogeneous and homogeneous catalysis. Theoretical and experimental studies demonstrated that particle mobility, which is governed by particle size and solution viscosity, could impact the intrinsic activity of the particle-attached enzymes (Jia et al., 2003).

As discussed above, most of the studies with nanoparticles have been dedicated to the improvement of enzyme activity and loading, rather than enzyme stabilization. In that regard, a recent report using magnetic nanoparticles for the enzyme immobilization is intriguing since a good enzyme stabilization was demonstrated with covalently attached lipase on the magnetic γ-Fe_2O_3 nanoparticles (Dyal et al., 2003). The final immobilization exhibited high stability for a month, and can be easily separated from the reaction medium by using a magnetic field. Interestingly, the specific activity (representing enzyme activity per unit mass of enzymes) of covalent-attached lipase on magnetic nanoparticles was lower than that reported for adsorbed enzymes on micrometer-sized polymeric beads. It was argued that the higher activity in the latter case was due to an overestimated enzyme activity since the enzyme desorbs from the beads to the reaction solution, leading to the increase of apparent enzyme activity by the enzyme reaction being carried out in solution rather than in a form of immobilized enzymes. To prevent this effect of leached enzymes