J|A|C|S COMMUNICATIONS

Published on Web 08/24/2009

Recyclable Nanobiocatalyst for Enantioselective Sulfoxidation: Facile Fabrication and High Performance of Chloroperoxidase-Coated Magnetic Nanoparticles with Iron Oxide Core and Polymer Shell

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Magnetic nanoparticles (MNPs) have received increasing attention in biorelated research due to several distinct properties such as high surface area to volume ratio, special magnetic behavior, and high dispersibility in various solvents.[1] An important application for MNPs is biocatalysis which has a profound impact on green and selective chemical syntheses. Enzyme-coated nanosized magnetic particles used as a nanobiocatalyst could be simply recycled under an external magnetic field. Compared with enzymes immobilized on micrometric supports, a nanobiocatalyst could achieve a much higher enzyme loading capacity and significantly enhanced mass transfer efficiency. An attractive approach for fabricating a nanobiocatalyst is to immobilize the enzyme on iron oxide MNPs which are cheap and biocompatible. Several enzymes such as hydrolase,[2] glucose oxidase,[3] and alcohol dehydrogenase[4] have been thus far immobilized on such MNPs.

The major problems in the fabrication of nanobiocatalysts at the current stage are the unsatisfied catalyst stability during operation and the recycling process and the dramatically reduced activity in comparison with free enzyme. Entrapment[5] and physical adsorption[2c] of enzyme on iron oxide MNPs suffered from the leakage of enzyme during reaction and recycling (loss of 50% activity after first cycle[2c]). Enzymes covalently immobilized on the surface of iron oxide MNPs showed unsatisfied stability (loss of 55% activity after five runs[3]), and these particles tended to aggregate.[3,2d] The use of silica encapsulated iron oxides MNPs for covalent enzyme attachment[6,2b] allowed us to increase the catalyst stability to some extent (loss of 35% activity after nine runs[2b]), but the obtained stability was not sufficient and the activity was only 22% of its free enzyme activity.[2b] Furthermore, the silica shell may contain pores and is unstable under alkaline conditions, the particle size distribution is difficult to control, and the synthetic methods are complicated and low-yielding.[1b] Here we report a new and simple method for fabricating an active and recyclable nanobiocatalyst by covalently immobilizing an enzyme on stable MNPs with a core containing multiple iron oxide MNPs and a thick polymer shell. Meanwhile, there is an increasing demand to develop efficient biocatalysts for asymmetric and green oxidations to prepare enantiopure fine chemicals. In this work, we focus on the first enantioselective oxidative nanobiocatalyst derived from chloroperoxidase (CPO).

The route for the preparation of the nanobiocatalyst is shown in Figure 1. Iron oxide particles (OA-MNPs) were prepared in 89% yield by a coprecipitation method with oleic acid as stabilizer.[7] A TEM image showed a mean size of OA-MNPs of 15 nm in diameter (Figure 2a), which also indicates that the particles are superparamagnetic. Glycidyl methacrylate (GMA) containing an epoxy functional group was then used as a monomer for *in situ* polymerization in the presence of OA-MNPs, giving 61% of new nanoparticles (GMA-MNPs) with a core-shell structure and uniform size distribution (Figure 2b). GMA-MNP has a mean size of ~90 nm, with a core in a diameter of 30 nm containing several OA-MNPs and a poly(GMA) shell with a thickness of 30 nm. This structure could effectively prevent the iron oxide core from leaching and further oxidation under harsh conditions. For obtaining better activity of the immobilized enzyme, ethylenediamine was used to prolong the bridge between the enzyme and MNPs. Reaction of GMA-MNPs with ethylenediamine gave the corresponding particles with amino groups (EDA-MNPs) in >99% yield. No dramatic shape or morphology change was observed during the modification due

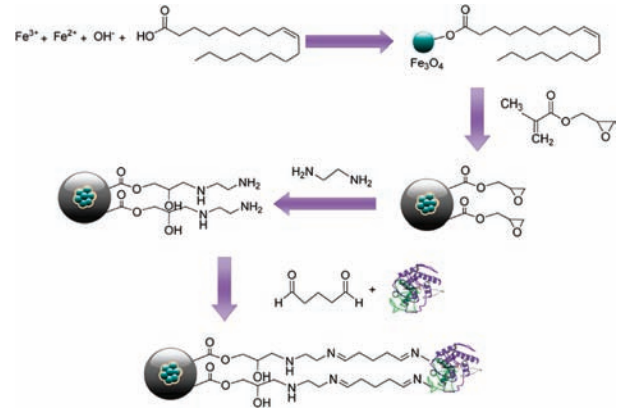

Figure 1. Synthetic route of active and stable magnetic nanobiocatalyst.

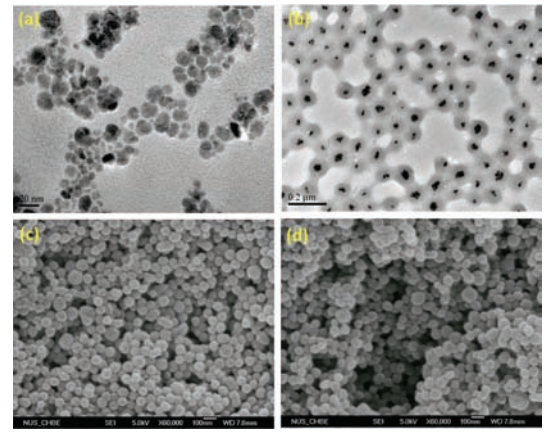

Figure 2. (a) TEM image of OA-MNPs. (b) TEM image of GMA-MNPs. (c) FESEM image of EDA-MNPs. (d) FESEM image of CPO-EDA-MNPs.

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10.1021/ja905477j CCC: $40.75

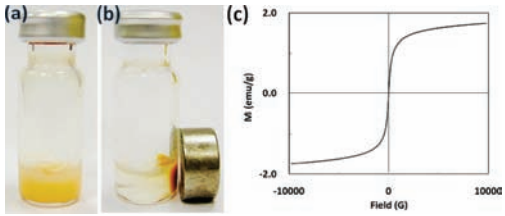

Figure 3. (a) CPO-EDA-MNPs in buffer. (b) Separation of CPO-EDA-MNPs by magnet after 2 min. (c) VSM of CPO-EDA-MNPs.

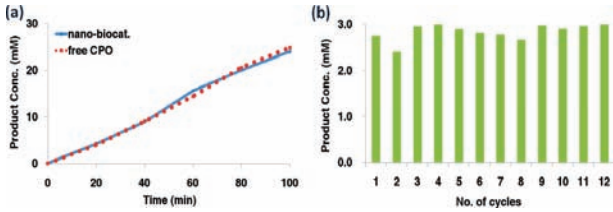

Figure 4. (a) Sulfoxidation of thioanisole by the nanobiocatalyst and free CPO. (b) Recycling and reuse of the nanobiocatalyst for the sulfoxidation.

to the dense polymer coating, and the resulting EDA-MNPs maintained spherical core-shell structures with a diameter of ~90 nm (Figure 2c).

An immobilization protocol was demonstrated by using CPO as the target enzyme. CPO is a versatile peroxidase for chemical synthesis including asymmetric oxidations, but it requires H_2O_2 as the oxidant which often causes significant enzyme deactivation. CPO has five amino groups from lysine residues, and three of them are located on the surface opposite to the active site.[8] Treatment of CPO, glutaraldehyde, and EDA-MNPs at pH 4.75 at room temperature resulted in covalent binding of CPO on the MNPs. A specific loading of 16.1 mg of CPO/g of MNPs was achieved for the nanobiocatalyst (CPO-EDA-MNPs) at optimized ratios of those reactants. This value is five times higher than the reported data using micrometric support via similar covalent binding.[9] From the FESEM images in Figure 2d, the morphology and size of CPO-EDA-MNPs did not change significantly after immobilization. Based on the composition and size of the MNPs, a single EDA-MNP could be estimated to have a weight of 6.54×10^{-16} g and a specific surface area of 49 $m^2\ g^{-1}$. The M_w of CPO is 50 kDa; thus ~126 CPO molecules are bound to one EDA-MNP (see Supporting Information). This corresponds to 12% occupation of enzymes on the particle surface area, similar to the value estimated on the basis of the data for a hydrolase directly immobilized on iron oxide MNPs.[10]

The magnetic property of CPO-EDA-MNPs was demonstrated in Figure 3a and 3b. The nanobiocatalyst was easily and quickly separated under a magnetic field. Vibrating sample magnetometry (VSM) in Figure 3c showed that CPO-EDA-MNPs exhibited superparamagnetic behavior at 298 K with a saturated magnetization value of 1.74 emu/g of particles.

Asymmetric sulfoxidation of thioanisole to (*R*)-methyl phenyl sulfoxide was chosen as the target reaction to investigate the catalysis and the recycling of CPO-EDA-MNPs. The courses of the sulfoxidation of 50 mM substrate with 50 mM H_2O_2 catalyzed by the nanobiocatalyst and free CPO, respectively, are shown in Figure 4a. In both cases, the product concentration increased linearly within 100 min, and no difference in catalytic performance was observed. The total turnover number of the nanobiocatalyst reached 25×10^3, which is also close to the reported data for free CPO in aqueous buffer.[11] In contrast, CPO immobilized on other solid materials resulted in a significant decrease of enzyme activity.[9,12] Moreover, the product ee was >99% (*R*) determined by chiral HPLC analysis. These results suggest no change in enzyme activity and enantioselectivity after covalent immobilization. In addition, the K_m of EDA-MNPs-CPO for monochlorodimedon was determined to be 26.1 μM, similar to the K_m of 27.7 μM for free CPO. This indicated again that there was no significant conformational change of the enzyme active site after immobilization.

Recycling of the nanobiocatalyst was conducted for the sulfoxidation of 5 mM substrate with 5 mM H_2O_2 for 10 min. After each cycle, CPO-EDA-MNPs were magnetically separated and added to the new reaction medium containing substrate and H_2O_2. The separation of the particles was easy and high yielding. Figure 4b showed the concentration of enantiopure (*R*)-sulfoxide produced in each cycle. After 12 cycles, the nanobiocatalyst was still fully active, thus being much better than CPO immobilized on other solid supporting materials.[9,12a]

In conclusion, a facile method for preparing MNPs comprising an iron oxide core, a polymer shell, and an enzyme-coated surface as a high performance nanobiocatalyst was developed. The covalently bound CPO with a long bridge showed the sulfoxidation activity and enantioselectivity to be the same as those for free CPO. The thick polymer shell significantly increased the stability of the nanobiocatalyst, giving no loss of activity after recycling 11 times. These results are much better than those achieved with CPO on other solid supports and represent the best performance on activity retaining as well as catalyst recycling among nanobiocatalysts known thus far. While it is the first example of a nanobiocatalyst for asymmetric oxidation, the new concept could be generally applicable for fabricating active and recyclable nanobiocatalysts.

Acknowledgment. Financial support from Singapore-MIT Alliance through the Flagship Research Projects in Chemical and Pharmaceutical Engineering Programme is acknowledged.

Supporting Information Available: Procedures for nanobiocatalyst synthesis, biotransformation, and catalyst recycling; analyses and characterizations of MNPs; calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA905477J