Introduction

Robust immobilization that preserves inherent activity of biocatalysts has been a technique of great importance for a range of applications in biotechnology including drug-delivery systems, biosensors, and enzymatic catalysis. A common strategy for immobilizing enzymes is their encapsulation inside sol–gel derived silica matrices, however, most studies showed lower specific activity than that of free enzymes due to the problems associated with slow reaction kinetics derived from its nonporous structure. Recent advances in the synthesis of nanostructured silicate materials via surfactant-induced self-assembly approaches particularly afford opportunities in terms of hosts or carriers for biocatalysts, because they are ideally suited to this task owing to the flexible tunability of structures and the high chemical stability [1]. A worthwhile challenge to this issue is to design high-performance heterogeneous biocatalysts combining high catalytic activity and improved recyclability and stability of enzymes, via a facile and scalable immobilization approach. We herein report a novel synthetic protocol for immobilizing enzymes retaining all of their activity and increasing the stability and recyclability [2]. The enzymes (Candida Antarctica lipase A (Cal-A)) were directly embedded within oil-filled spherical silica nanoparticles (OSN) having core–shell structure via an anionic surfactant-induced self-assembly approach, and the resultant enzyme–silica composites (Cal-A@OSN) were demonstrated to act as efficient heterogeneous biocatalysts in both aqueous and organic solvent.

Materials and Methods

The Cal-A@OSN composite was synthesized via an anionic surfactant-induced self-assembly approach using oleic acid, where the enzymes were directly added into the initial synthesis medium under ambient conditions to circumvent the denaturation of enzymes, and silicon sources (tetraethoxyorthosilicate and 3-aminopropyl triethoxysilane) were subsequently added to yield protective mesoporous silica shells. The structures were investigated by using SEM, TEM, XRD, and nitrogen adsorption–desorption measurements. The enzymatic activity of the enzyme–silica composites were assessed by hydrolysis reaction of esters in water and transesterification reaction of triglycerides in n-heptane.

Results and Discussion

The SEM and TEM images of Cal-A@OSN verify that it is composed of monodispersed spherical silica nanoparticles with an average particle size of ca. 230 nm whose cavity spaces are filled with oleic acid (Figure 1a and 1b). XRD and N$_2$ adsorption measurements identified nonporous structure of as-synthesized Cal-A@OSN, but confirmed the existence of 1-D wormhole-like mesopores for the calcined sample. From a fluorescence microscopy image, it was disclosed that enzymes were selectively sequestered within the silica matrix of the outer shell, not in the oil phase. These combined analyses verify a formation of enzyme–silica composites with hierarchical core–shell structure as illustrated in Figure 1c.

The Cal-A@OSN composite exhibited as high a reaction rate as that of free enzyme in hydrolysis of ester in buffer solution, indicating excellent retention of its enzyme activity upon immobilization. This is mainly due to the combination of the ambient synthetic conditions that preserve the inherent activity of enzymes and an optimized configuration of enzymes in the vicinity of the reactive surface that maximize the contact between enzymes and reactants. More significantly, the enzyme activity was at least up to 10 times reproduced while retaining more than 97% of the initial activity in each cycle, and was mostly retained over wide temperature and pH ranges, demonstrating its enhanced recyclability and stability. The fine reusability and thermal/chemical stability of enzymes can be attributed to the robust immobilization ability of the silica matrix of the shell. Furthermore, Cal-A@OSN could be used as an efficient heterogeneous biocatalyst in hydrolysis and transesterification reactions of triglycerides in an organic solvent over multiple reaction cycles, whereas enzyme itself is insoluble in the organic media. It is believed that the oil (oleic acid)-induced organophilicity of OSN increases the affinity with the organic media and leads to a prominent catalytic activity.

Significance

In comparison with the prototypical composite biocatalysts without a hierarchical structure, the enzyme immobilization protocol developed in this study provides several advantages: i) mild synthetic conditions that preserve the intrinsic activity of enzymes, ii) direct entrapment of enzymes via a facile preparation route which simplify the manufacturing process, iii) the protection effect endowed by the silica shell which allow for increased stability and recyclability of enzymes, and iv) the organopholicity properly induced by the oil in the core, which allows for efficient catalysis particularly in organic solvent. With those favourable characteristics, this method will be a promising approach for synthesizing high-performance heterogeneous biocatalysts combining improved recyclability and stability of enzymes.

References