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Immobilization of *Candida Antarctica* lipase B in a silicified hydrogel support and its application as bioreactor

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Abstract

Supramolecular hydrogels have attracted increasing interest in recent years because of their ability to incorporate high levels of proteins, cells, antibodies, peptides and genes [1-2]. In this work, we propose a new approach to confinement of Candida Antarctica lipase B (CALB) within a supramolecular silicified hydrogel based on Pluronic F127 and α-cyclodextrin (α-CD) [3]. After functionalization of the matrix, the catalytic performance of the supported biocatalyst was evaluated in the oxidation of 2,5-diformylfuran (DFF) to 2,5-furandicarboxylic acid (FDCA), a fully biosourced alternative to terephthalic acid used in the production of polyethylene terephthalate (PET) [4]. Our results revealed that while CALB immobilized in conventional sol-gel silica yielded exclusively 5-formylfuran-2-carboxylic acid (FFCA), confinement of the enzyme in the silicified hydrogel imparted a 5-fold increase in DFF conversion and afforded 67% FDCA yield in 7 h and almost quantitative yields in less than 24 h. The hierarchically interconnected pore structure of the host matrix was found to provide a readily accessible diffusion path for reactants and products, while its flexible hydrophilic-hydrophobic interface was extremely beneficial for the interfacial activation of the immobilized lipase.

Supramolecular hydrogels with a three-dimensional cross-linked macromolecular network have attracted growing scientific interest in recent years because of their ability to incorporate high loadings of bioactive molecules such as drugs, proteins, antibodies, peptides, and genes. Herein, we report a versatile approach for the confinement of *Candida antarctica* lipase B (CALB) within a silica-strengthened cyclodextrin-derived Supramolecular hydrogel and demonstrate its potential application in the selective oxidation of 2,5-diformylfuran (DFF) to 2,5-furandicarboxylic acid (FDCA) under mild conditions. The enzymatic nanoreactor was deeply characterized using thermo gravimetric analysis, Fourier transform infrared spectroscopy, N_2-adsorption, dynamic light scattering, UV–visible spectroscopy, transmission electron microscopy, scanning electron microscopy, and confocal laser scanning microscopy, while the reaction products were established on the basis of ^1H nuclear magnetic resonance spectroscopy combined with high-performance liquid chromatography. Our results revealed that while CALB immobilized in conventional sol–gel silica yielded exclusively 5-formylfuran-2-carboxylic acid (FFCA), confinement of the enzyme in the silicified hydrogel imparted a 5-fold increase in DFF conversion and afforded 67% FDCA yield in 7 h and almost quantitative yields in less than 24 h. The hierarchically interconnected pore structure of the host matrix was found to provide a readily accessible diffusion path for reactants and products, while its flexible hydrophilic–hydrophobic interface was extremely beneficial for the interfacial activation of the immobilized lipase.

In this study, Candida antarctica lipase B (CALB) was immobilized onto ECR1030 resin and the obtained immobilized preparation was used for the synthesis of n-3 polyunsaturated fatty acids (PUFA)-rich triacylglycerols (TAG). The immobilization process was systematically studied. Under the optimized conditions, the immobilized preparation of ECR1030-CALB with an esterification activity of 10058 U/g was obtained, which was comparable with the commercially available Novozym 435. Confocal microscopy images showed that CALB diffused from the surface to the center of carrier during immobilization. The basic properties of ECR1030-CALB were also investigated and it was found that the thermostability, acidic and alkaline stability, and organic solvent tolerance of ECR1030-CALB were comparable with Novozym 435. Interestingly, ECR1030-CALB showed significantly higher specificity towards EPA and DHA compared with Novozym 435, which made it suitable for the synthesis of n-3 PUFA-rich TAG. The TAG content of 74.05% was obtained under the optimized conditions, which was slightly higher than that (73.68%) obtained by Novozym 435. This is the first study for systematically studying the immobilization process of lipase using ECR1030 resin as carrier. Overall, the prepared ECR1030-CALB with excellent esterification activity, basic properties, and catalytic performance might be a promising alternative to commercial Novozym 435. Practical applications: A previous study found that ECR1030 resin was a robust and promising carrier for the immobilization of CALB. However, the detailed immobilization conditions, the basic properties, and catalytic performance of the immobilized preparations using ECR1030 resin as carrier are still unknown. Consequently, knowledge of the above unknown information for the immobilization of CALB using ECR1030 resin as carrier is of great importance for their further practical applications in lipid chemistry.

Candida antarctica lipase B (CALB) was immobilized on the macro porous resin by physical adsorption in organic medium.

The immobilization was performed in 5 mL isooctane, and the immobilization conditions were optimized. The results were achieved with the mass ratio of lipase to support 1:80, the buffer of pH 6.0, initial addition of PBS 75 microL, and immobilization time of two hours at 30 degrees C. Under the optimal conditions, the activity recovery was 83.3%. IM-CALB presented enhanced pH and thermal stability compared to the free lipase, and showed comparable stability with the commercial Novozym 435, after 7 times repeated use for catalyzing the synthesis of ethyl lactate, 56.9% of its initial activity was retained, and only 24.7% was retained when used for catalyzing the hydrolysis of olive oil.