doi:10.2533/chimia.2017.345 Chimia 71 (2017) 345–346 © Swiss Chemical Society

Surface Immobilization and Shielding of a Transaminase Enzyme for the Stereoselective Synthesis of Pharmaceutically Relevant Building Blocks

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Abstract: Transaminases are enzymes capable of stereoselective reductive amination; they are of great interest in the production of chiral building blocks. However, the use of this class of enzymes in industrial processes is often hindered by their limited stability under operational conditions. Herein, we demonstrate that a transaminase enzyme from *Aspergillus terreus* can be immobilized at the surface of silica nanoparticles and protected in an organosilica shell of controlled thickness. The so-protected enzyme displays a high biocatalytic activity, and additionally provides the possibility to be retained in a reactor system for continuous operation and to be recycled.

Keywords: Chiral building blocks · Transaminases

1. Introduction

Transaminases, also called aminotransferases, are enzymes that catalyze the transfer of an amino group from a donor to an acceptor (keto acid, aldehyde or ketone) with the help of a vitamin B_6-cofactor (pyridoxal 5'-phosphate).[1] As this reductive amination reaction occurs in a stereoselective fashion, it allows for the synthesis of a broad range of chiral amines (Fig. 1).

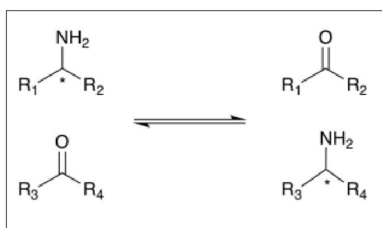

Fig. 1. Transaminase-catalyzed transamination.

Working on the development of biocatalytic (nano)materials, we have recently developed a method to produce active and stable nanobiocatalysts.[2] The chemical strategy developed relies on the controlled growth, at the surface of silica nanoparticles where an enzyme is covalently immobilized, of a protective layer of controlled thickness. Atomic force microscopy experiments demonstrate that the protective organosilica shell produced at the surface of the carrier material undergoes a softening process yielding a soft yet protective environment for the immobilized enzyme.[2a] The mechanical properties and chemical composition of the protective layer can be adjusted to the enzyme to be protected and to the targeted application.[2b] The so-shielded enzyme displays outstanding stability vs. physicochemical (freeze/thaw cycles, ultrasound, heat), chaotropic (urea) and biochemical (protease) stress conditions. Herein, we demonstrate that this method of enzyme protection can be adapted to a transaminase enzyme.

2. Experimental

The carrier SNPs were produced as previously described.[3] All chemicals, solvents, enzymatic assay kit and enzymes were purchased from Sigma (Switzerland). A detailed procedure for the production of immobilized and protected enzyme has been published by us for a β-galactosidase.[4] We have adapted this procedure to the selected transaminase. Scanning electron microscopy experiments were carried out using a Zeiss SUPRA® 40VP scanning electron microscope. A 2 μL drop of the sample was spread on freshly cleaved mica substrates, dried, and sputter-coated with a gold-platinum alloy. Electron micrographs were acquired with an accelerating voltage of 20 kV using the InLens mode. Particle size analyses were carried out using the Olympus® AnalySIS software package.

3. Results and Discussion

As carrier material for the immobilization/shielding of the enzyme, we used silica nanoparticles (SNPs) produced using the Stöber method.[5] This method has the advantage of yielding monodisperse particles, which are required for the layer growth analysis. The nanoparticles were further modified with 3-aminopropyl-triethoxysilane (APTES); the use of a limited reaction duration (30 min) and low concentration of APTES allows SNPs to be produced with only a limited number of amine functions and leaving free silanol functions at the surface of the SNPs.[3] This step is crucial to allow the further covalent attachment of the protective organosilica layer at the surface of the SNPs. As model enzyme, we chose a commercial fungal ω-transaminase from *Aspergillus terreus.* This enzyme was immobilized on amino-modified silica nanoparticles with a diameter of 310 nm. The bio-conjugation reaction was carried out using glutaraldehyde as cross-linker. We optimized the reaction parameters by varying the concentration of enzyme and by measuring the amount of non-immobilized enzyme at the end of the coupling reaction using the established bicinchoninic acid (BCA) assay (Fig. 2).

The results presented in Fig. 2 showed that, in the studied conditions, the highest concentration for the transaminase immobilization is 100 μg mL^{-1} (equivalent to 31 μg of protein per mg of SNPs). This value is reached with an initial concentration of 200 μg mL^{-1}, which represents an immobilization yield of 50%. It is noteworthy that this immobilization yield can be improved by increasing the amount of amino functions at the surface of the SNPs. However, this might negatively impact the number of free silanol functions and thus hamper the formation of the protective organosilica shield.

The SNPs displaying immobilized transaminase at their surface were further reacted with a mixture of APTES and tetra-ethyl-orthosilicate (TEOS), the latter serving as a precursor of inorganic silica.[2a] The SNPs were thoroughly washed and analyzed

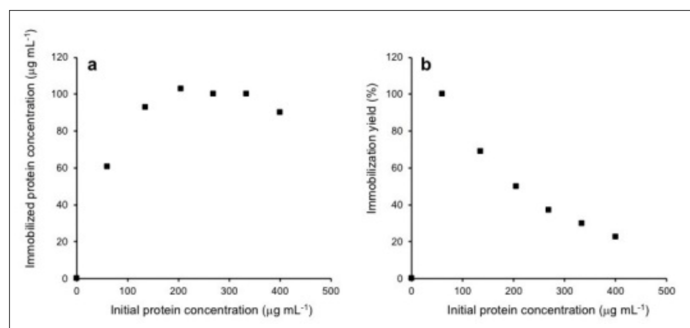

Fig. 2. Optimization of enzyme immobilization. a) Concentration of immobilized protein vs. initial protein concentration and b) immobilization yield in function of initial protein concentration ([immobilized protein]/[initial protein] x 100).

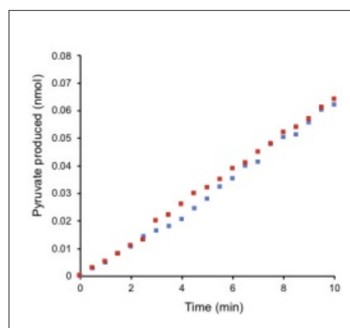

Fig. 4. Enzyme kinetics study. Pyruvate production using shielded (■) and soluble (■) transaminases (equivalent to 20 μg mL^{-1} of enzyme)

with field-emission scanning electron microscopy (FE-SEM); a representative micrograph is shown in Fig. 3.

The FE-SEM revealed particles with a smooth surface and a diameter of 376 ± 10 nm. This represents an increase of 32 nm with regard to the starting 'naked' particles (344 ± 10 nm in diameter). The layer shielding the enzyme is thus of 16 nm. Considering the diameter of the enzyme of 7 nm, measured from the crystal structure of the enzyme,[6] the immobilized enzyme is

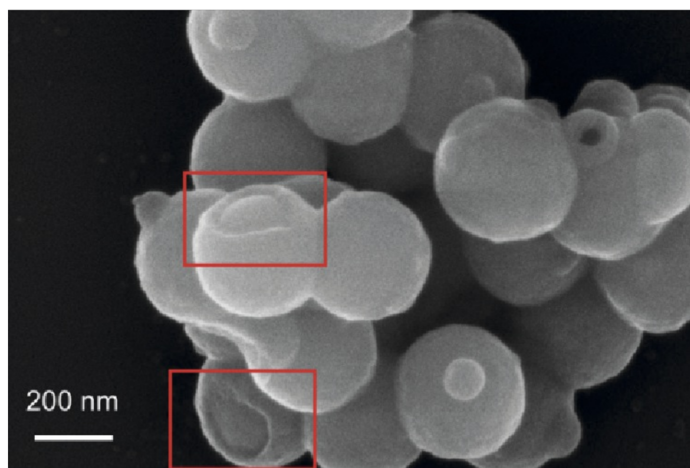

Fig. 3. Representative FE-SEM micrograph of SNPs with immobilized transaminase enzyme further shielded in an organosilica layer of 33 nm. The red squares highlight areas where the organosilica layer did not grow (certainly owing to aggregation with another SNPs) and where the silica core is visible.

fully buried in the organosilica layer.

The enzymatic activity of the SNPs produced has been measured using a transaminase-specific assay based on the reversible transfer of an amino group from alanine to α-ketoglutarate, generating pyruvate and glutamate (Fig. 4).

The results of enzyme kinetics shown in Fig. 4 clearly demonstrated that the presence of the organosilica shield does not hamper the enzymatic reaction. In the test conditions, the reaction velocity of the shielded enzyme was identical to that of the soluble control (at the same concentration).

4. Conclusion

In this short communication, we demonstrate that a transaminase enzyme, namely a transaminase from *Aspergillus terreus*, can be immobilized at the surface of silica nanoparticles and shielded in an organosilica protective layer. The activity measurements of this enzyme indicated that in the selected reaction conditions, the immobilization and shielding of the enzyme did not cause any significant enzyme denaturation. Additionally, the nano-porosity of the shielding organosilica layer did not limit substrate/product diffusion through this layer. The work is underway to study the use and recyclability of the produced nanobiocatalysts in process-relevant conditions.

Received: March 21, 2018

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