Immobilizing transaminase in a macroporous silica monolith for flow mode enantioselective transamination

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1. Introduction

The use of enzymes as catalysts in the chemical sector is extremely appealing but their application in industrially relevant processes is not straightforward. In fact, the use of free enzymes in homogeneous batch processes suffers from severe limitations, some which can be tackled using immobilization strategies. To go even further towards green processes, the design of continuous flow production processes is a relevant strategy.[1] In this context, beside enzyme immobilization, catalyst shaping is a crucial aspects.

ω-transaminases (TA) are currently attracting a great deal of attention for the greener production of chiral amines, which are precursors of many important drugs.[2] In this communication, we will present the immobilization of TA on silica monoliths that are particularly appropriate for the design of continuous flow reactors (Fig. 1A). The silica monoliths (Fig. 1B-E) are prepared by a bottom-up sol-gel method based on emulsion templating.[3] They minimize pressure drop, ensure a plug flow regime, and are easy to manipulate.

After presenting the proof-of-concept[4] with a simple flow reactor featuring covalently immobilized TA (ATA-117 from Codexis), we will show how the immobilization of the enzyme – and therefore the catalyst performance – can be optimized by tuning parameters of the monolith surface functionalization. We will show that a self-produced TA of higher purity allows reaching higher production rates. We will also disclose an attempt to run a chiral amine synthesis using a cascade biocatalytic process to shift the thermodynamic equilibrium of the reaction towards the desired product, as proposed by Turner et al.,[5] but here in flow mode.

2. Experimental

A His-tagged TA was produced in the lab from the plasmid (GeneScript) via expression in E. coli and purification. Macrocellular self-standing silica monoliths were prepared using an emulsion-based sol-gel protocol[6] and then functionalized using either glycidoxypropyltrimethoxysilane (GPTMS) or aminopropyltriethoxysilane (APTES) to bring epoxy or amino groups on the surface. Materials are characterized by TGA, ATR-IR, N_2-physisorption, SEM. A heat-shrinkable Teflon tube was used to cast the monolith and design a simple flow reactor.[4] Enzyme was loaded in flow mode using the Ni^{2+}/NTA/His-tag complexing strategy or by covalent grafting using glutaraldehyde (Fig. 2). The transamination of pyruvate with racemic 4-bromo-α-methylbenzylamine (BMBA) to produce bromoacetophenone (BAP) was used as a model reaction (kinetic

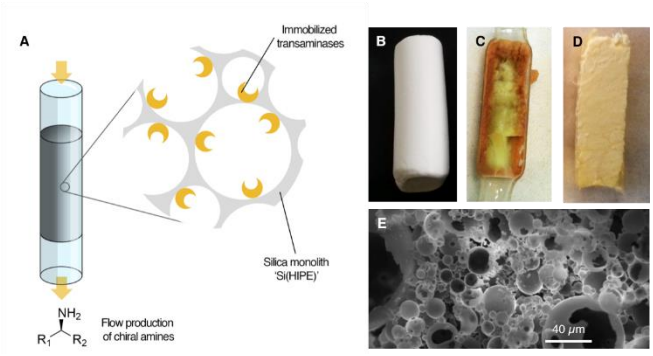

Figure 1. (A) Flow strategy for the synthesis of chiral amine using TA immobilized in a macrocellular silica monolith. (B) Picture of the silica monolith. Longitudinal cut in the monolith showing poor (C) and excellent (D) APTES dispersion. (E) SEM micrograph of the monolith

resolution). Then, the enantioselective synthesis of R-BMBA from BAP and alanine was studied, using the same TA, together with co-immobilized glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH).

3. Results & Discussion

The biocatalysts obtained through covalent grafting of the commercial enzyme with APTES showed good performance in the model reaction, with high repeatability.[4] By adjusting the contact time, full conversion can be obtained. The solid biocatalyst was fully enantioselective, stable, recyclable, and showed no diffusional limitations. In the next steps we investigate important improvements toward the implementation of a flow biocatalytic process for the synthesis of chiral amines.

First, a poor APTES dispersion in the monolith was macroscopically observed (Fig. 1C) and confirmed by TGA and IR analyses. This was assigned to the functionalization mode in which excess APTES in the solution accumulates at the outer layers of the monolith, causing an egg-shell dispersion. Parameters known to affect the dispersion have been controlled in order to get better dispersion of amino groups and subsequently better catalytic properties: both wet and dry impregnation methods were tested for the silanization step. Preparation was conducted with a fine control on silanol (silica re-hydroxylation) and water availability (use of dried monolith and water saturated solvent). It turned out that controlling the water content during the functionalization step allows obtaining a homogeneous APTES dispersion (Fig. 1 D) and this was correlated with an increase in the activity of the monoliths by a factor 3.

Second, the purity of the commercial enzyme was questioned and we also wanted to investigate the possibility to immobilize the enzyme through a His-tag. The enzyme produced in the lab showed much higher purity and intrinsic activity. Yet, when immobilized using the His-tag/NTA/Ni^{2+} strategy (Fig. 2), only poor levels of conversion were obtained. On the contrary, when immobilized via the lysine residues using the optimized APTES grafting and glutaraldehyde, conversion was increased by a factor 4 as compared to the commercial enzyme.

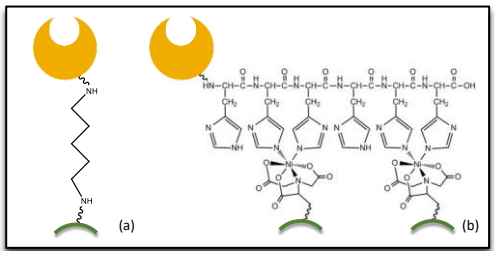

Fig. 2. Immobilization strategy using (a) APTES + glutaraldehyde or (b) GPTMS + NTA + Ni^{2+} + His-tag

Third, it is known that the thermodynamic equilibrium of the reaction is strongly shifted towards the consumption of the desired chiral amine (only ~0.5% conversion of bromoacetophenone is expected if the transamination alone is considered). The batch mode cascade reaction showed that it was indeed possible to consume the by-product (pyruvate) and obtain significant production of R-BMBA when combining TA with GDH and LDH. The same strategy was attempted in the flow-mode using the three enzymes co-immobilized on the same monolith. We show that the grafting order is important and that the proportions of each enzyme should be chosen carefully to take into account the intrinsic reactivity of the respective enzymes.

4. Conclusions

The effective immobilization of enzymes on macrocellular supports that can withstand flow conditions opens up new perspectives for the design of efficient biocatalytic processes, especially relevant for the synthesis of high added value chemicals using highly engineered enzymes.

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