

Room-temperature solid phase ionic liquid (RTSPIL) coated ω -transaminases: Development and application in organic solvents

B. Grabner^{a,b,*}, M.A. Nazario^a, M.T. Gundersen^b, S. Loïs^c, S. Fantini^c, S. Bartsch^d, J.M. Woodley^b, H. Gruber-Woelfler^{a,*}

^a Institute of Process and Particle Engineering, Graz University of Technology, Inffeldgasse 13/III, 8010 Graz, Austria

^b Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 227, 2800 Kgs., Lyngby, Denmark

^c Solvionic SA, Site Bioparc SANOFI, 195 route

^d Espagne - BP1169, 31036 TOULOUSE Cedex 1, France

ARTICLE INFO

Keywords:

Biocatalysis
Transaminase
Organic solvent
Ionic liquid
RTSPIL

ABSTRACT

ω -Transaminases ATA-40, ATA-47 and ATA-82P were coated with room-temperature solid phase ionic liquids (RTSPILs) by means of three methods, melt coating, precipitation coating, and co-lyophilization, and showed increased stability in all of the five tested organic solvents. Co-lyophilization and melt coating were further found to have an activating effect on the enzymes. The former led to an up to 8-fold increase of reaction rate and excellent recyclability. The coating also protected the cofactor pyridoxal 5'-phosphate (PLP), which is essential for transaminase activity, from degradation, leading to a reaction proceeding for 27 days. With this method the sparingly water soluble substrate 5-bromo-1-indenone could be processed enzymatically in cyclohexane as solvent.

1. Introduction

Transaminases are highly potential enzymes to assist in the synthesis of molecules of interest to both the chemical and pharmaceutical industry [1–4]. Their products, chiral amines, are of high value and possess a wide range of applications such as intermediates for fine chemicals and resolving agents for enantiomeric resolution [5]. Since 40% of active pharmaceutical ingredients (APIs) are estimated to contain a chiral amine, enantiomerically pure amines take a remarkable position in pharmaceutical industry [6,7]. An exemplary biocatalytic industrial process employing an ω -transaminase (EC 2.6.1.X) is the total synthesis of Sitagliptin, the API of Januvia® [8]. Sitagliptin is according to the BCS (Biopharmaceutics Classifications System) a Class III (high solubility/low permeability)/borderline Class I compound used to treat Type 2 diabetes mellitus [9]. In this case, the high water solubility of the compound makes a biocatalytic process in aqueous solution feasible.

However, not all APIs belong to this class. In fact, almost half of the drugs on the market contain a poorly water soluble API and 80–90% of pipeline drugs are poorly water soluble [10–12]. Combining Class II (low solubility/high permeability) and Class IV (low solubility/low permeability) compounds with biocatalysts in aqueous systems is therefore challenging. Such systems are limited to low concentrations of

hydrophobic substrate, generate large quantities of waste and make downstream processing (DSP) more difficult [13]. In this case, the use of organic solvents as an alternative reaction media can make a process economically viable [14]. On the other hand, enzymes often are inactivated by organic solvents, resulting in poor reaction rates, relative to those observed in aqueous media. To overcome this problem, various techniques for immobilization and stabilization of enzymes in organic solvents have been investigated in the past decade [15,16]. One of the approaches includes the coating with ionic liquids (ILs). In 2002, Lee and Kim were the first to coat an enzyme with an ionic liquid [17]. Applying RTSPIL (room-temperature solid phase ionic liquids) with a melting point slightly above room-temperature enabled the use of enzymes as a heterogeneous catalyst in a solid/liquid system. These ionic liquid-coated enzymes (ILCE) showed increased enantioselectivity. This technique for immobilizing enzymes in ionic liquids was investigated by various researchers in the past decade. Mostly lipases were coated with various ILs or pre-incubated in ILs prior the reaction, resulting in excellent enantioselectivity and increased reaction rate [18–30]. Coating enzymes with RTSPIL is an excellent supplement to biocatalysis in RTIL (room-temperature ionic liquids) where the ILs are used as a solvent [31–41].

In 2010, Lee and Kim, reported an alternative method to stabilize enzymes in organic solvents – ionic liquid co-lyophilized enzymes

* Corresponding authors at: Institute of Process and Particle Engineering, Graz University of Technology, Inffeldgasse 13/III, 8010 Graz, Austria.

E-mail addresses: b.grabner@tugraz.at (B. Grabner), woelfler@tugraz.at (H. Gruber-Woelfler).

were found to be enantioselective and up to 660-fold more active than their free counterpart [42]. Since then, co-lyophilized enzymes (mostly lipases) with ILs have been investigated only by few researchers [43,44].

We present here a novel approach to coat transaminases with ILs. To the best of our knowledge transaminases have so far not been tested in combination with IL coating, but were already applied in organic media and two-phase systems [2,14,45,46]. This article will show the applicability of ionic liquid coating on cofactor dependent transaminase. The coated enzymes showed extended stability in various organic solvents, especially in cyclohexane. Enzyme coated by co-lyophilization additionally showed increased reaction rate compared to the free enzyme.

2. Results and discussion

2.1. Preliminary studies

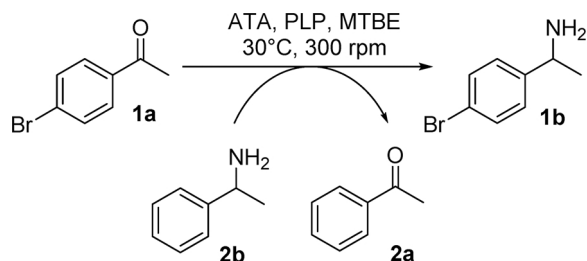
In preliminary studies two coating methods were tested and the activity of the coated and the free enzymes were compared using methyl-*t*-butyl-ether (MTBE) as organic solvent. After defining the requirements of ILs for coating enzymes that require water in their reaction mechanism, namely (i) a melting point above room and operation temperature, (ii) a melting point low enough to not inactivate the enzyme during the melt coating process, (iii) water miscibility or at least insensitivity to water, and (iv) insolubility in the used solvent, a list of potential ILs was compiled. Amongst others, 1-ethyl-3-methyl-imidazolium bromide [Emim]Br (EB) meets these requirements and was chosen for coating transaminase ATA-40 applying two different methods, melt coating and precipitation coating. The melt-coated enzyme was produced by heating the IL above its melting point and mixing the melt with the enzyme and cofactor. For the precipitation-coated enzyme, the IL was precipitated out of a concentrated aqueous solution of the IL, enzyme and cofactor.

The two prepared solid phases were then used for the enzymatic reductive amination of 4'-bromoacetophenone **1a** with methylbenzylamine **2b** as amine donor forming the product 1-(4-bromophenyl)ethanamine **1b** and the co-product acetophenone **2a**. (Scheme 1)

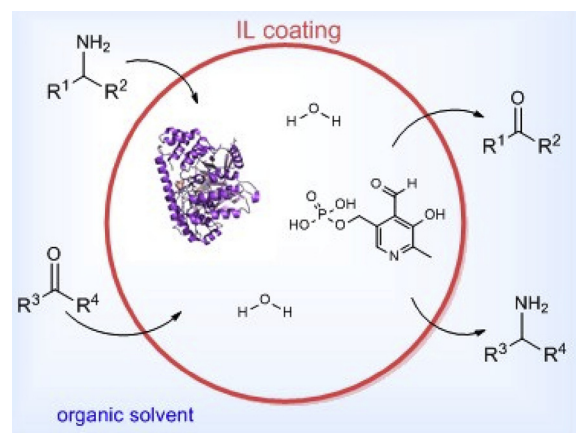
The reaction system is assumed to work as shown in Scheme 2. The IL coating encloses the catalytic system consisting of the enzyme, PLP and water, protecting it from the organic solvent. The substrates dissolved in the organic phase need to pass the IL barrier in order to reach the active site and undergo a reaction. Afterwards, the formed products diffuse back into the organic solvent.

As a reference, a reaction with soluble free enzyme was also carried out. Samples were taken over time and analyzed by means of GC. Because of its low volatility the product could hardly be analyzed by means of GC. Therefore, the co-product (**2a**, acetophenone) concentration was observed over time. (Fig. 1)

At the beginning of the reaction, the soluble free enzyme run fastest with an initial rate of $50.8 \mu\text{mol L}^{-1} \text{h}^{-1}$ (X in Fig. 1), but it slows down and finally stops after less than 30 h of reaction. Addition of further cofactor restarted the reaction for 50 h, but after 120 h the reaction rate



Scheme 1. Enzymatic amination of **1a** employing ATA-40 in MTBE using **2a** as donor resulting in product **1b** and co-product **2b**.



Scheme 2. Scheme of the reaction system. Enzyme, PLP and water are enclosed in the IL coating. The substrates need to diffuse through the coating to reach the active site. The products diffuse back into the surrounding organic.

decreased again and the reaction subsequently stopped. In contrast, coating the enzyme and the cofactor with IL melt (○ in Fig. 1) stabilized the reaction over 120 h indicated by the constant slope (Fig. 1).

The reaction rate of the melt coated enzyme is with $18.2 \mu\text{mol L}^{-1} \text{h}^{-1}$ lower than that of the initial reaction rate of the soluble free enzyme. This might be due to the additional barrier the substrate needs to overcome to reach the active site of the enzyme or potentially due to reduced activity caused by the coating process itself. The behavior of the precipitate coated enzyme (□ in Fig. 1) is almost the same as for the melt coated catalyst. The reaction rate of $4.9 \mu\text{mol L}^{-1} \text{h}^{-1}$ is approximately constant during the entire experiment. As with the melt-coated catalyst, the reaction proceeds over 120 h.

However, compared to the melt-coated enzyme, the reaction runs at a lower rate. This reduction in reaction rate was thought to be caused by the coating process and/or the added acetone for the precipitation. Therefore, acetonitrile was used as anti-solvent in the later experiments because residuals of acetone can participate in the reaction and act as amino acceptor. However, later experiments showed, that the coating method itself is less efficient, independent from the used anti-solvent. Another possibility for the loss of activity is the way enzyme and cofactor are immobilized on/in the IL crystals. If the cofactor is too far from the enzyme and cannot diffuse to the active site, less active enzyme-PLP complexes can form and less turnover per time unit can happen.

To find out how the coating thickness affects the reaction rate, enzymes were coated with various amounts of IL (1:1, 1:2, 1:5, and 1:10 in terms of enzyme mass).

Using 1:2 of IL for the melt-coating process decreases the reaction rate compared to the 1:1 coating. It is likely that this effect is due to a hindered mass transport through the coating. For all higher amounts of coating material almost no conversion was observed. Indeed, the coating may get too thick for the substrate to permeate.

Coating the enzyme with double the amount of IL with the precipitation method hardly effects the initial reaction rate. However, thicker coatings (1:5 and 1:10) result in very low catalytic activity.

Interestingly, coating only the enzyme, by melt or precipitation coating, and adding the cofactor in solution with the substrate did not result in any reaction. This observation can either be caused by the inability of the cofactor to permeate through the coating or the low probability of a substrate and PLP molecule to permeate and bind to the enzyme simultaneously.

2.2. Solvent stability

Starting from this point, and knowing about the stabilizing effect of the coating, we decided to investigate the method of coating enzymes

Download English Version:

<https://daneshyari.com/en/article/8916786>

Download Persian Version:

<https://daneshyari.com/article/8916786>

[Daneshyari.com](https://daneshyari.com)