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Enantioselective transesterification of secondary alcohols mediated by aminoacylases from *Aspergillus* species

M. Bakker *, A.S. Spruijt, F. van de Velde, F. van Rantwijk, R.A. Sheldon

Laboratory of Organic Chemistry and Catalysis, Julianalaan 136, 2628 BL Delft, Netherlands

Abstract

The aminoacylases (*N*-acyl-L-aminoacid amidohydrolase; E.C. 3.5.1.14) from *Aspergillus melleus* and *Aspergillus oryzae* catalyze the enantioselective transesterification of 1-phenylethanol with absolute stereospecificity. Increased catalytic efficiencies were obtained by using solubilized surfactant-coated aminoacylase complexes, which makes them more attractive for industrial application. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Enantioselective transesterification; Secondary alcohols; *Aspergillus* species

1. Introduction

Aminoacylase (*N*-acyl-L-aminoacid amidohydrolase; E.C. 3.5.1.14) from fungal sources is used industrially in the enantioselective hydrolysis of *N*-acetyl aminoacids [1]. Its catalytic capabilities are much wider, however, and include transesterification [2,3]. The most commonly used aminoacylases are those from Porcine kidney, *Aspergillus oryzae*, and *Aspergillus melleus* [4,5]. We have now compared the enzymes from *A. melleus* and *A. oryzae* with regards to their catalytic efficiency in the enantioselective transesterification of 1-phenylethanol (**1**) in organic media (Scheme 1).

The use of freely suspended enzyme lyophilisates in organic media is increasingly regarded as inefficient. Solubilisation, which presents a solution for the incompatibility of enzymes and organic media, has been performed by extraction with lipophilic counterions [6], coprecipitation with an amphiphilic compound [7], and colyophilisation with a polymer [8] or a surfactant [9]. It has also been demonstrated that colyophilisation with a crown ether has a spectacular activating effect on proteases, such as subtilisin, upon use in organic media [10].

We now report that colyophilisation with surfactants very considerably enhances the catalytic activity of aminoacylases in an anhydrous solvent-free reaction medium.

2. Materials and methods

2.1. Materials

Aminoacylase from *A. melleus* (E.C. 3.5.1.14) and Bradford reagent were purchased from Sigma. Aminoacylase from *A. oryzae* was a gift from Degussa (Hanau, Germany). Polyvinylpyrrolidone K30 (PVP), Aerosol OT (10 % v/v) (AOT), (*R*,*S*)-**1**,

* Corresponding author. Tel. : +31-15-278-2683; fax: +31-15-278-1415.

E-mail address: secretariat-ock@tnw.tudelft.nl (M. Bakker).

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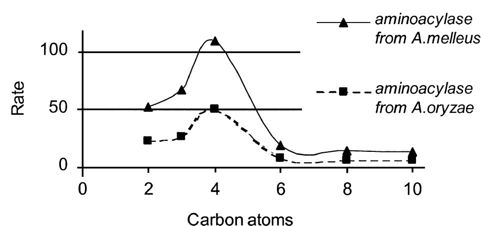


Fig. 1. Effect of the chain length of the acyl donor on the transesterification rate of **1**

vinyldecanoate, and *N*-acetyl-L-methionine were obtained from Aldrich. Cumeen, vinyl acetate, and vinyl propionate were purchased from Acros. Vinylbutyrate was obtained from Fluka; vinyl hexanoate and vinyloctanoate were from Tokyo, Kasei, Kogyo.

2.2. Analysis and equipment

The lyophilization of enzyme-surfactant solutions was performed in rubber sealed freeze-dry flasks (Salm en Kipp bv, 300 ml). Reversed phase HPLC analysis was performed using a custom-packed Symmetry C_{18} cartridge (Waters Radial-Pak, 8×100 mm, 7 μm) contained in a Waters RCM 8×10 compression unit, with simultaneous detection on a Waters 486 tunable absorbance detector with Waters Millenium[32] software. The products of *N*-acetyl-L-methionine hydrolysis were analyzed using acetonitrile-phosphate buffer (50 mM; pH 2.2) 7.5:92.5 (v/v) as eluent (flow 1.5 ml/min for the first 4 min followed by 3 ml/min), with detection at 210 nm. The products of transesterification of **1** were analyzed on chiral HPLC using a Chiralcel OD column (Daicel Chemical Industries, 250×4.6 mm), eluent flow 0.6 ml min^{-1}, using cumene as internal standard, isopropanol-hexane 5:95 (v/v) as eluent and detection at 254 nm. Optical rotations were measured using a Perkin Elmer 241 polarimeter. UV measurements were performed on a Cary 3 spectrophotometer from Varian.

2.3. Enzyme lyophilization

To prepare surfactant coated protein, 5 g of dry weight PVP K30 was dissolved in 50 ml buffer (100 mM KH_2PO_4, pH 8.0) and mixed with 1 g of catalyst. After achieving homogeneous enzyme solution, the liquid was frozen at −40°C and the resulting frozen aqueous layer was removed under vacuum during 16 h. The activity of aminoacylase preparations was measured using the standard hydrolysis of *N*-acetyl-L-methionine as the assay [11].

In the case of AOT, 28 g of emulsion (10 % Aerosol) was mixed with 50 ml buffer (100 mM KH_2PO_4, pH 8.0) and when 1 g of enzyme was dissolved, the liquid was frozen and dried under vacuum.

2.4. Transesterification reactions

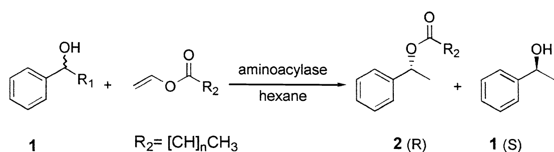
Experiments to investigate the effect of the chain length of the acyl donor on the transesterification rate were performed by shaking, at room temperature, 100 mg of **1** (0.8 mmol) with 1.5 equivalent acyldonor ($n = 0, 1, 2, 3, 5, 7, 9$) (1.2 mM), 100 mg catalyst and 5 ml hexane (Fig. 1). During the first 5 h, the initial rate was measured by following the course of the reaction with chiral HPLC.

With the surfactant-coated catalyst the reactions were performed without additional solvents by solubilizing 200 mg of surfactant-prepared catalyst in 3 g **1** (24.6 mmol) and adding 1.1 equivalents vinyl butyrate (27 mmol) was added. The samples for analysis were centrifuged to remove the surfactant/

Table 1
Properties of the aminoacylases from *A. oryzae* and *A. melleus*

Source	Protein content[a] (mg protein g^{-1})	Hydrolytic activity[b] (u/mg protein)	Transesterification (initial rate; μmol min^{-1} g^{-1})
A. oryzae	85	11.2	51
A. melleus	45	10.0	110

[a] Bradford assay.
[b] One unit will hydrolyse 1 μmol *N*-acetyl-L-methionine/min.



Scheme 1. Transesterification of 1-phenylethanol (**1**)

protein layer from the substrate/product. When the reaction was complete, the enzyme-complex was separated from the reaction mixture by centrifugation. The excess of vinyl butyrate was evaporated and the residue was purified by silica gel column chromatography (ethylacetate/petroleumether 1:1) to afford (*S*)-**1**-alcohol and (*R*)-**2**-ester.

3. Results and discussion

3.1. Comparison of *A. melleus* and *A. oryzae* enzymes

Both enzyme preparations contained only a minute amount of active protein (Table 1). The catalysts acted highly enantioselectively ($E > 500$) in the acylation of **1** with different vinyl esters (Scheme 1).

With vinyl butyrate, a 50 % conversion of **1** was reached within 30 h and (*S*)-**1** and (*R*)-**2** esters were obtained in quantitative yield with both catalysts. Although the specific hydrolytic activity of the enzyme from *A. oryzae* was slightly higher (Table 1), the initial rate in the transesterification reaction was markedly higher for *A. melleus* aminoacylase.

The effect of the chain length of the acyl donors on the rate of the acyl transfer was studied next. Both catalysts showed optimum activity with vinyl butyrate ($n = 2$, Fig. 1). The *A. melleus* enzyme was twice as active as the one from *A. oryzae* for all acyl groups investigated. A longer chain length of the acyl donor resulted in a decrease of the reaction rate by a factor six with a correspondingly longer reaction time for complete conversion of (*R*)-**1**.

3.2. Surfactant coated protein complexes

In order to increase the catalytic efficiency, we investigated the use of solubilized surfactant-coated catalyst. Aminoacylase complexes were prepared by lyophilizing the catalyst with the surfactants AOT and PVP. The enzyme-PVP complex was soluble in the reaction mixture whereas the AOT complexes formed a biphasic system. The transesterification of **1** with vinylbutyrate was performed without additional solvent (Table 2). The decrease in initial rate compared with the results in Table 1 indicates that inhibition by the alcohol **1** takes place.

The AOT-enzyme complexes were much more active transesterification catalysts than the PVP complexes, although both preparations had a similar hydrolytic activity. The AOT-complex with the *A. oryzae* aminoacylase was much more active in transesterification than the one with *A. melleus* enzyme;

Table 2
Surfactant-aminoacylase complexes as transesterification catalyst

Aminoacylase source	Hydrolytic activity[a] (u/mg protein)	Transesterification (initial rate; μmol min^{-1} g^{-1})	STY (g l^{-1} day^{-1})	Productivity[b] (g g^{-1} h^{-1})
A. oryzae				
Native	11.2	0.2	45	0.6
PVP-complex	3.89	2.4	70	6.4
AOT-complex	4.38	74	2104	127.2
A. melleus				
Native	10.0	0.4	47	1.2
PVP-complex	6.22	3.6	50	8.8
AOT-complex	5.33	20.0	376	41.1

[a] One unit will hydrolyse 1 μmol *N*-acetyl-L-methionine min^{-1}.
[b] Productivity in g product g^{-1} protein h^{-1}.

we note that with the native enzymes, the reverse was true (Table 1). With the AOT complex of the *A. oryzae* aminoacylase, a quantitative conversion of (*R*)-**1** was achieved in 4 h. After work-up, a yield of 42 % of pure ester **2** was achieved [+(**2***R*): $[\alpha]_D =$ +81.8 ($CHCl_3$, $c = 1.2$); −(**1***S*): $[\alpha]_D = -45.0$ ($CHCl_3$; $c = 1.0$)].

Lyophilization of the purified aminoacylases from both sources with surfactants resulted in preparations with much lower activity (data not shown). Apparently, the adjuvants exert a stabilizing effect on the protein, as has also been suggested by other workers [12]. It is also relevant to note that colyophilisation with salts increased the catalytic activity of proteases suspended in organic solvents [13].

The different results that we obtained with complexes of the aminoacylases from *A. melleus* and *A. oryzae* could be caused by differences in the amino acid residues on the surface.

We note that the aerosol complex of the aminoacylase from *A. oryzae* shows a considerable potential for industrial biocatalysis. The availability and low cost of the enzyme coupled with high volume yields, high productivities, and space time yields [2 kg l^{-1} day^{-1} for (*R*)-**2**-ester and 1.3 kg l^{-1} day^{-1} for (*S*)-**1**-alcohol] make our procedure very attractive for practical use in enantioselective transesterifications.

4. Conclusion

The aminoacylases from *A. melleus* and *A. oryzae* discriminate the enantiomers of **1** very efficiently. A very high space-time yield was accomplished using an aminoacylase-AOT complex as catalyst in a solventless system.

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