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Enantioselective acylation of chiral amines catalysed by aminoacylase I

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Abstract—Aminoacylase (E.C. 3.5.1.14) from *Aspergillus melleus* mediated the acylation of the primary amino group in a range of primary arylalkylamines and amino alcohols in anhydrous organic medium. The commonly used vinyl and isopropenyl esters proved to be unsuitable acyl donors because rapid uncatalysed aminolysis occurred in the presence of these additives. The unwanted aminolysis reaction could be suppressed by performing the enzymatic reaction in *tert*-butyl methyl ether medium with methyl 2-methoxyacetate as the acyl donor. We found that chiral amines were acylated with poor to moderate enantioselectivity, in contrast with the quantitative enantiodiscrimination that is commonly observed with the corresponding alcohols. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Aminoacylase I (*N*-acyl-L-amino acid amidohydrolase, E.C. 3.5.1.14) from *Aspergillus* sp. is a readily available and inexpensive enzyme with a relaxed substrate tolerance.[1] It is used in the industrial production of enantiopure L-amino acids from their *N*-acyl derivatives.[2,3] The related aminoacylase from porcine kidney also mediates the reverse reaction, the acylation of L-amino acids,[4,5] in organic medium. Herradón et al. subsequently showed that aminoacylase from *Aspergillus melleus*, in anhydrous organic medium, efficiently catalyses the—irreversible—acyl transfer from vinyl acetate to alcohols and amines.[6,7] Further investigations revealed that secondary arylalkanols are acylated with high, often near-absolute, enantioselectivity[8,9] and a productivity surpassing that of the corresponding lipase-catalysed reactions.[9]

The use of aminoacylase as an amine acylation catalyst has received scant attention,[4–6] despite the fact that enantiopure amines are of considerable interest both as chiral building blocks and auxiliaries. We present herein a study on the enantioselective acylation of a variety of arylalkylamines using a range of acyl donors.

2. Results and discussion

We initially attempted to acylate representative chiral amines, such as 1-aminoindane **1** (see Fig. 1), with vinyl acetate or butyrate, which has successfully been used in the resolution of chiral alcohols.[8,9] It became clear that the uncatalysed aminolysis of this activated ester was undesirably fast, in particular at synthetically relevant concentrations;[10] isopropenyl esters and acetoxyacetone could not be used for the same reason. Hence, a range of prospective acyl donors was subjected to enzymatic as well as uncatalysed aminolysis by **1**; the reaction rates are compiled in Table 1. In general, the cat/uncat ratio increased with decreasing activity of the donor, but a very low acyl transfer rate, such as observed with methyl butanoate, was evidently undesirable. Methyl

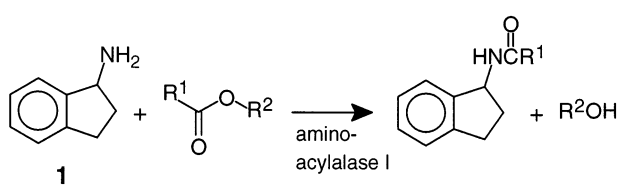

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Figure 1. Acylation of 1-aminoindane (**1**).

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Table 1. Effect of the acyl donor on the aminoacylase I-mediated acylation of 1-aminoindane **1**[a]

Acyl donor	Initial rate ($\mu M\ min^{-1}$)	Ratio cat/uncat
Methyl butanoate	0.04	>300
Allyl acetate	0.2	>300
Ethyl 2-(methylamino)acetate	0.3	>300
Ethyl 2-methoxyacetate	0.6	120
Methyl 2-methoxyacetate	1.7	140
2,2,2-Trifluoroethyl butanoate	1.8	10
Acetoxyacetone	5.5	<1

[a] Reaction conditions: **1** (50 mM), acyl donor (60 mM), aminoacylase I (50 U), hexane (5 mL), rt.

2-methoxyacetate[11] provided the best compromise and was used in all subsequent experiments; ethyl 2-methoxyacetate and 2-(methylamino)acetate could also be useful acetyl donors.

We next investigated the effects of changing the reaction medium on the acylation of **1**. It has been shown that hydrophobic solvents, such as hexane or toluene, are the preferred reaction media for aminoacylase I-catalysed transesterification,[8,9] but the limited solubility of many reagents of interest in such hydrophobic solvents seriously limits their practical utility. We performed the acylation of **1** by methyl 2-methoxyacetate in a variety of solvents and found that the initial rate correlates linearly with log *P* over a wide range (see Fig. 2). In contrast, the nature of the solvent had a negligible effect on the enantiodiscrimination of **1** as well as 1-amino-2-indanol **2** and 1-phenylethylamine **3** (see Table 2). *tert*-Butyl methyl ether (TBME) was selected as a compromise solvent because it adequately dissolved the reagents of interest with only a small decrease in reaction rate.

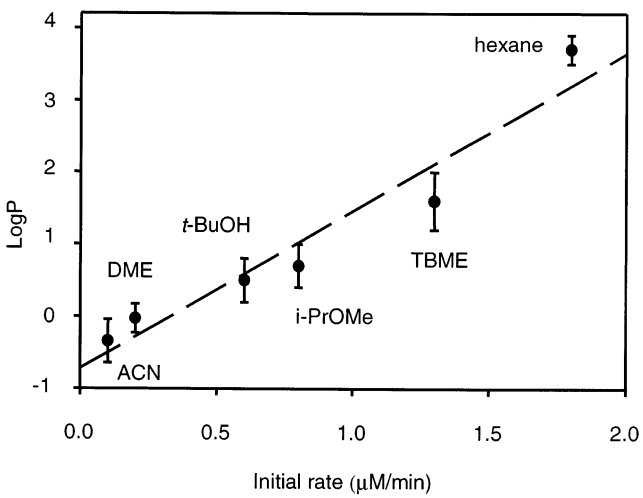

Figure 2. Acylation of 1-aminoindane by methyl 2-methoxyacetate in the presence of aminoacylase I; relationship of initial rate and log *P*. Abbreviations: ACN: acetonitrile, DME: 1,2-dimethoxyethane, *t*-BuOH: *tert*-butyl alcohol, *i*-PrOMe: isopropyl methyl ether, TBME: *tert*-butyl methyl ether.

Table 2. Acylation of chiral amines **1** by methyl 2-methoxyacetate mediated by aminoacylase I. Effect of the reaction medium on the enantiomeric ratio[a]

Amine	Solvent (*E*)		
	Hexane	TBME	1,2-Dimethoxyethane
1	9.7	9.3	10.2
2	2.7	3.0	3.4
3	7.8	8.4	8.0

[a] Reaction conditions: amine (50 mM), acyl donor (60 mM), aminoacylase I (50 U), solvent (5 mL), rt.

A variety of arylalkylamines (**1**–**8**, see Fig. 3) was acylated in the presence of aminoacylase I under these conditions. Compounds **1**, **3**, **6** and **8**, which had only an amine functionality, were resolved with moderate enantioselectivity ($E = 7$–9, see Table 3). In contrast, the same enzyme mediated the acylation of 1-phenylalkanols with near-absolute enantioselectivity.[9] 1-(1-Naphthyl)ethylamine reacted much more slowly than the other compounds, which is presumably due to steric hindrance from the bulky naphthyl substituent.

The series also included three amino alcohols **2**, **4** and **7** to assess the effects of a primary hydroxyl group on the chemo- and enantioselectivity of the biocatalyst. We found that the selectivity for amino acylation was absolute but the enantioselectivity was very poor. The enantiomers of phenylglycine methyl ester **5** were also poorly discriminated.

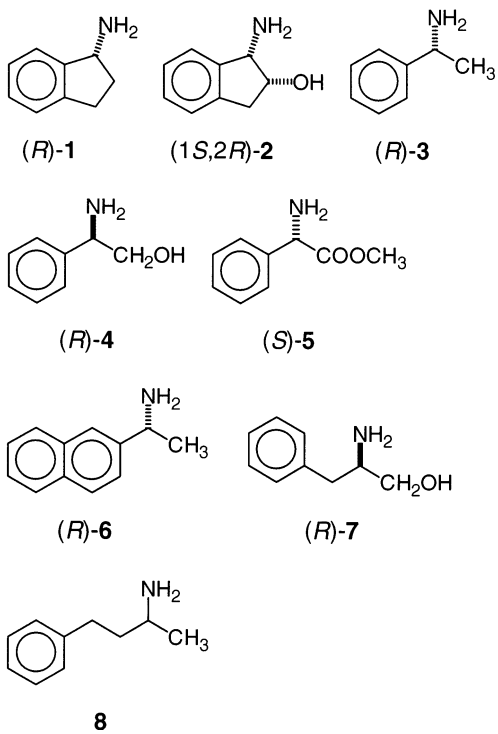

Figure 3. Structures of amines **1**–**8** showing the preferentially converted enantiomer.

Table 3. Enantioselective acylation of arylalkylamines in the presence of aminoacylase I[a]

Compound	Initial rate (μM h^{-1})	Preferred enantiomer	Conversion (%)	E.e.$_{amine}$ (% *S*)	*E*[b]
1	47	*R*	53	72	9.3
2[c]	4	1*S*,2*R*	37	26	3.0
3	34	*R*	57	78	8.4
4	37	*R*	46	12	1.3
5	18	*S*	52	56	5.4
6	3	*R*[d]	27	28	9.1
7	47	*R*	54	24	1.8
8	35	Nd	47	56	7.3

[a] Reaction conditions: amine (15 mM), methyl methoxyacetate (20 mM), aminoacylase I (50 U), MTBE (5 mL), rt.
[b] Determined via numerical integration of e.e.$_S$ versus ξ (see Section 4).
[c] Reaction in 1,2-dimethoxyethane.
[d] Tentatively assigned on the basis of the HPLC retention time (see Section 4).

The difference in enantiodiscrimination of secondary arylalkanols and the corresponding amines is particularly striking and difficult to explain. Presumably, alcohols and amines bind differently in the active site. Recent work has indicated that aminoacylase I also has aminopeptidase activity[12] and, hence, it seems plausible that amines could also bind in the subsite that, in the normal reaction pattern, receives the *N*-terminal group.

The reaction temperature can profoundly affect the steric course of enzymatic reactions[13] and a temperature decrease often,[14–16] but not universally, results in improved stereoselection. Thus, we investigated the effects of the reaction temperature on rate and enantioselectivity of the acylation of **1**–**3**. As we expected, the acylation rate decreased steeply as the temperature was lowered. The enantiomeric ratio improved only by a factor of 2, or less, for a 36°C temperature decrease, which is in the same range as we previously observed with lipase-mediated resolutions[15,16] but is too low to be of practical significance.

3. Conclusions

Aminoacylase I from *A. melleus* mediates the acylation of primary arylalkylamines with low to moderate enantioselectivity. The amino group is preferentially acylated in the presence of a primary alcohol function.

4. Experimental

4.1. Materials

Aminoacylase I from *A. melleus*, 1.3 U mg^{-1}, was purchased from Fluka. The activity of aminoacylase was measured using the standard hydrolytic assay of *N*-acetyl L-methionine.[9] One unit (U) will liberate 1 μmol of L-methionine per min.

Allyl acetate, 1-aminoindane, (*R*)-(−)-2-amino-2-phenylethanol, (*S*)-(+)-2-amino-2-phenylethanol, (*S*)-(−)-2-amino-3-phenyl-1-propanol, (*R*)-(+)-2-amino-3-phenyl-1-propanol, 2-amino-4-phenylbutane, 2-heptylamine, isopropenyl acetate, ethyl methoxyacetate, methyl methoxyacetate, *N*-acetyl-L-methionine, racemic 1-phenylethylamine, (*R*)-(+)-1-phenylethylamine, (*S*)-(−)-1-phenylethylamine, were purchased from Acros, Belgium. Acetoxyacetone, (*R*)-(−)-1-aminoindane, *S*-(+)-1-aminoindane, (1*S*,2*R*)-(−)-1-amino-2-indanol, (1*R*,2*S*)-(+)-1-amino-2-indanol, methyl butyrate, 2,2,2-trifluoroethyl butyrate, vinyl acetate, vinyl butyrate were purchased from Fluka. Racemic 1-(1-naphthylethylamine was from Lancaster, England. (*R*)- and (*S*)-Phenylglycine methyl esters were kindly donated by DSM Fine Chemicals, Netherlands. The organic solvents and buffer compounds were commercial products of analytical or HPLC grade.

4.2. Analysis and equipment

The progress of the reactions was monitored by reversed phase HPLC chromatography using a Chrompack 4.6×150 mm 5 μ Nucleosil C-18 column, with detection on a Waters 486 tunable absorbance detector with Waters Millenium[32] software. The hydrolysis of *N*-acetyl-L-methionine was monitored using acetonitrile–aqueous 50 mM phosphate buffer pH 3.0 (15:85, v/v) as the eluent at a flow rate of 0.5 mL min^{-1} and detection at 210 nm. The mobile phase for monitoring the acylation of the amines and aminoalcohols was prepared by adjusting a 0.68 g L^{-1} solution of KH_2PO_4 in acetonitrile–water (30:70, v/v) containing 0.68 g L^{-1} sodium dodecylsulphate to pH 3.0 with phosphoric acid. The flow rate was 0.5 mL min^{-1}; detection at 210 nm. The retention times of the products were compared with those of chemically prepared samples.

The enantiomeric purity of the unreacted amines **1**–**7** was analyzed by chiral HPLC using a 150×4 mm Crownpak CR(+) column, with detection on a Waters 486 tunable absorbance detector at 215 nm with Waters Millenium[32] software (Table 5). The eluants used and retention times found are compiled in Table 4. The enantiomeric purity of the acylation product of **8** was analyzed using 4.6×250 mm Chiralcel OD column, eluant hexane–*iso*-propyl alcohol (95:5, v/v) at a flow rate of 0.8 mL min^{-1}.

Table 4. Temperature effects on rate and enantioselectivity[a]

Amine	Initial rate (μM h^{-1})			E		
	4°C	20°C	40°C	4°C	20°C	40°C
1	16	47	120	13	9.6	9.1
2[b]	1.5	4.3	4.5	5.0	3.4	2.5
3	12	34	100	10.0	8.2	7.8

[a] Reaction conditions: amine (15 mM), methyl methoxyacetate (20 mM), aminoacylase I (50 U), MTBE (5 mL), rt.
[b] Reaction in 1,2-dimethoxyethane.

4.3. Acylation of amines

Acylations were carried out in 10 mL closed reaction vessels at the appropriate temperature under permanent stirring. The reactants were dissolved and the reaction was initiated by adding 50 U aminoacylase I. Further details are given in the table footnotes. Non-enzymatic (blank) reactions were performed under the same conditions but without enzyme. Samples were withdrawn in the course of the reaction, centrifuged, and subjected to HPLC analysis as described above.

4.4. Calculation of the enantiomeric ratio

It is common practice to calculate E from a single datapoint by applying Sih's equations.[17] We have sought to increase the accuracy and reliability by calculating E via numerical integration of Eq. (1) with subsequent regression analyses.[18] Thus, E was determined by fitting the experimental point sets of $e.e._S$ versus ξ (where $e.e._S$ is the enantiomeric excess, and ξ is the conversion of the amine, see Fig. 2) by numerically integrating Eq. (1) with minimization of the sum of squares of deviations as criterium (Fig. 4).

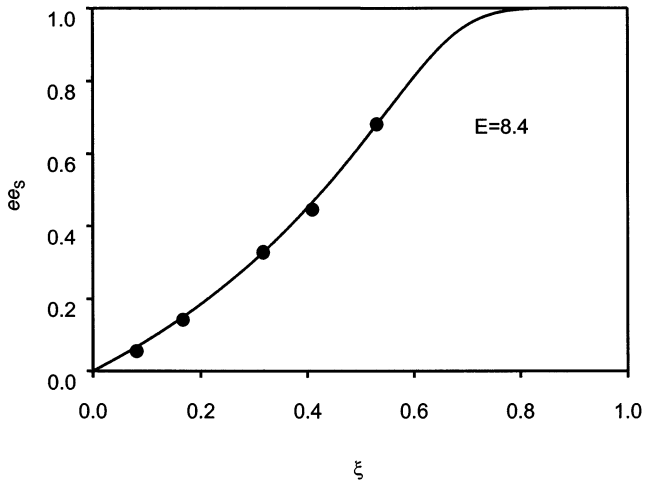

Figure 4. Eq. (1) fitted through the datapoints of the acylation of **3** at 20°C to calculate E. Abbreviations: ξ: conversion of **3**, $e.e._S$: enantiomeric excess of **3**.

$$\frac{de.e._S}{d\xi} = \frac{(1+e.e._S)(1-e.e._S)}{(1-\xi)\left(\frac{E+1}{E-1} - e.e._S\right)} \quad (1)$$

Acknowledgements

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Table 5. Chiral HPLC of **1**–**7**

Amine	Column	Mobile phase	Flow rate (mL min^{-1})	Retention time (min)	
				R	*S*
1	Crownpak CR(+)	Aqueous $HClO_4$ pH 1, 20°C	0.6	60	40
2	Crownpak CR(+)	Aqueous $HClO_4$ pH 1, 20°C	0.6	15[a]	18[b]
3	Crownpak CR(+)	Aqueous $HClO_4$ pH 1, 20°C	0.6	40	29
4	Crownpak CR(+)	Aqueous $HClO_4$ pH 1, 20°C	0.6	23	15
5	Crownpak CR(+)	Aqueous $HClO_4$ pH 2, 20°C	0.6	18	37
6	Crownpak CR(+)	MeOH–aqueous $HClO_4$ pH 2 (5:95, v/v), 40°C	0.6	135[c]	120[c]
7	Crownpak CR(+)	aqueous $HClO_4$ pH 1, 20°C	0.6	27	30

[a] (1*R*,2*S*).
[b] (1*S*,2*R*).
[c] Configuration tentatively assigned on the basis of the retention time.

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