ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 11 (2001) 487–490

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Bis-phenacetyl and phenoxyacetyl groups as substrates for penG and penV amidases

Cinzia Barbieri, Enrico Caruso, Paola D'Arrigo, Sara Frattini, Giuseppe Pedrocchi-Fantoni, Stefano Servi *

CNR, Centro di Studio sulle Sostanze Organiche Naturali, Dipartimento di Chimica, Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy

Abstract

o-, *m*-, *p*-Bis-phenylacetic and -bis-phenoxyacetic acid esters with solketal are prepared and submitted to enzymatic hydrolysis with penicillin V (PVA) and G (PGA) amidases. While the *para*-isomers are recovered unchanged, *ortho*- and *meta*-bis-esters are completely hydrolysed. PVA shows a reversed substrate specificity, hydrolysing phenylacetates faster than its natural substrate. The use of the bis-acids as alcohol-protecting groups is proposed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Penicillin V amidase; Penicillin G amidase; Bis-phenylacetates; Bis-phenoxyacetates; Substrate specificity; Enzymatically removable protecting group

1. Introduction

Bicarboxylic acids have been used as protecting groups in the synthesis of nonracemic chiral compounds. Besides the function of temporarily masking a reactive functional group (–OH), they can become the linkers of two chiral moieties consisting of the two enantiomeric forms of a chiral alcohol in nonequal amounts, thus generating diastereoisomers. In the course of the synthesis, the intermediate diastereoisomers can be eventually crystallised, thus allowing to improve the enantiomeric purity of the compound after removal of the linker/protecting group [1]. Terephtalates [1], phtalates and oxalates [2] have been used for the purpose.

Due to the availability and specificity of certain hydrolytic enzymes, the use of enzymatically cleavable protecting groups has also been proposed. In particular, penicillin G amidase (PGA) has been used for the selective cleavage of phenylacetic acid esters in the presence of other functionality of similar chemical reactivity [3]. Thus, enzymatically removable protecting groups consisting in bicarboxylic acids could be of interest in synthetic applications of nonracemic chiral compounds. The possibility of using such a type of protecting group has not been reported so far.

We considered bis-phenoxyacetic acid esters and bis-phenylacetic esters as possible enzymatically removable bifunctional protecting groups for the masking of alcohol and amine functionality in esters and

* Corresponding author. Tel.: +390-22-399-3047; fax: +390-22-399-3080.

E-mail address: servi@dept.chem.polimi.it (S. Servi).

1381-1177/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(00)00148-X

amides. Phenoxy- and phenylacetic acid esters are the substrates of penicillin V amidase (PVA) and PGA, respectively. Both bacterial enzymes have been developed for the preparation of 6-APA from penicillin V (penV) and G (penG), respectively, and have been in industrial use for this purpose for years. Although they act on similar substrates, they have apparently quite different mass and sequence. PVA (EC 3.1.5.11) has been isolated from several microorganisms, *Bacillus sphaericus* and *Fusarium oxysporum*, among others. Comparison of the specificity of PGA from *E. coli* and PVA is available but only with the PVA from *B. sphaericus* whose crystal structure has recently been solved. PVA from *B. sphaericus* hydrolyses penG and other phenylacetates at a rate of 1/10 of the natural substrate, penV. However, the relatively high K_m values observed for PVA from a variety of bacterial sources for the natural substrates suggests that the enzyme has a rather loose fit hence being prone to the recognition of nonnatural substrates [4–6]. The observation that PVAs from *B. sphaericus* and from *Fusarium* show high rates of hydrolysis only when both the side chains of the substrate and the 6-APA moiety are present in the molecule [6] partly contradicts the previous statement. Notwithstanding these apparently strict structural requirements, this enzyme, like many others, can be of use in the hydrolysis of nonnatural substrates, although at a much lower rate.

In this article, we describe the hydrolysis rates of the isomeric phenylacetates and phenoxyacetates of the same primary alcohol (solketal). The data will eventually add some information about the substrate specificity of the two enzymes and will tell the applicability of those acids as enzymatically removable alcohol-protecting groups.

2. Materials and methods

PGA immobilised on Eupergit C® with a specific activity of 60 U/g was a gift from Recordati (Milano, Italy).

PVA Semacylase type A, batch PPV 1856, was from Novo. It consisted of glutaraldehyde cross-linked cells of *F. oxysporum*. It had about 25 U/g of wet material. A total of 1 U of activity was assigned prior to use for the hydrolysis of 1 μmol/min of the natural substrate at 30°C and pH 7.6.

2.1. Bis-phenylacetyl chloride and bis-phenoxyacetyl chloride

A total of 10 g of bicarboxylic acid was suspended in 120 ml of thionyl chloride. The mixture was refluxed until all the substrate was dissolved and then for 1 h longer. The excess thionyl chloride was removed by distillation and the products crystallised from toluene–hexane mixtures.

*2.2. Bis-phenylacetyl and bis-phenoxyacetyl esters of isopropylidene glycerol (solketal) **1–3***

A total of 1.5 g of solketal was dissolved in 20 ml of methylene chloride and 3 ml of anhydrous pyridine. The temperature was maintained at 0–10°C while 3.8 g of acyl chloride was added in small portions. The mixture was then stirred at room temperature for 12 h. The organic phase was partitioned with water and a $NaHCO_3$ saturated solution and finally evaporated to give the crude ester in nearly quantitative yield.

2.3. Bis-phenylacetyl and bis-phenoxyacetyl amides of phenylethylamine

To a solution of 2 g of acyl chloride in 60 ml of methylene chloride, 2 ml of anhydrous triethylamine and 2.3 g of phenylethylamine were added at room temperature. The mixture was stirred overnight, washed with water and a saturated $KHSO_4$ solution. From the resulting organic phase, the amides were recovered with nearly quantitative yield.

*2.4. Hydrolysis of bis-phenylacetyl and bis-phenoxyacetyl esters **1–3** with PVA*

A total of 150 mg of PVA (3.75 U) in 25 ml of water was adjusted at pH 7.5 with 0.1 M NaOH and 50 mg of substrate in 2.5 ml of acetonitrile was added. The reaction was monitored by titration with 0.02 M NaOH.

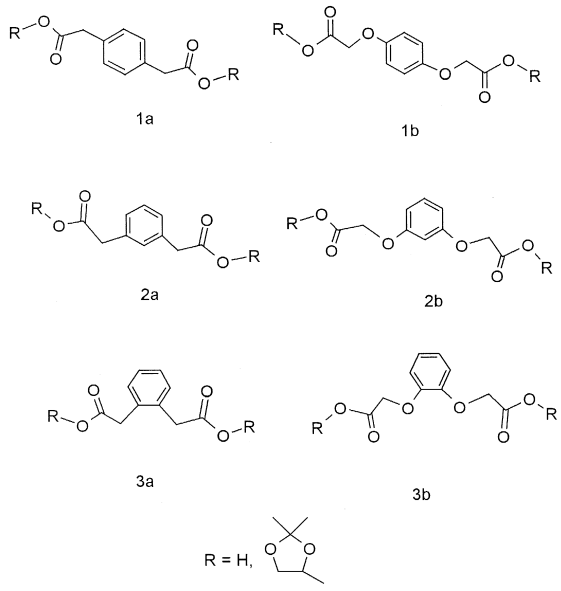

Fig. 1. Substrates for PVA and PGA catalysed hydrolysis.

2.5. Hydrolysis of bis-phenylacetyl and bis-phenoxyacetyl esters *1–3* with PGA

A total of 50 mg of PGA (3 U) in 10 ml of water was adjusted at pH 7.6 with 0.1 M NaOH and 50 mg of substrate in 2.5 ml of acetonitrile was added. The reaction was monitored by titration with 0.02 M NaOH.

2.6. Hydrolysis of bis-phenylacetyl and bis-phenoxyacetyl amides with PGA and PVA

The hydrolysis was carried out as described in Sections 2.4 and 2.5 for the corresponding esters (Fig. 1).

3. Results and discussion

From the six isomeric bicarboxylic acids, prepared though conventional methods, the corresponding esters **1–3** with enantiomerically enriched solketal were obtained. The esters can be used in a multi-step bidirectional-synthesis not requiring terminus differentiation, as already shown for the corresponding phtalates [1]. In the present study, removal of the protecting group was directly checked on substrates **1–3**. It is known that the substrate specificity for the two enzymes resides on the acid part, at least when esterified with alcohols relatively small if compared to the natural substrates [7]. On the other hand, it has been reported that PGA will not accept *p*-substituted phenylacetic acid derivatives as substrates, due to restrict binding possibility for this type of compounds. The same kind of information are not available for PVA.

This tendency is confirmed by the data concerning the enzyme catalysed hydrolysis of the two *p*-substituted esters **1a** and **1b** (Table 1). They are recovered unchanged with PGA. PVA hydrolysis of the two compounds had a significant initial rate of 0.8 and 0.6 μmol/min, respectively, but the reaction came at an end around 20% conversion. This is presumably an effect of product inhibition. Since the enzyme catalysed reaction employed about 3.7 U of PVA, the observed rate is only five times slower than the reaction on the natural substrate. The two substrates **a** and **b** are transformed at a comparable rate. PGA hydrolysis proved useful only on the *o*-disubstituted phenylacetate **3a** which was transformed almost completely in 48 h at an initial rate of 1.1 μmol/min. PVA hydrolysed all the substrates at different rates: compound **2a** was completely reacted within 48 h, whereas the best substrate among the phenoxyacetate esters was the compound with the *ortho* substitution pattern **3b**. It is surprising that PVA hydrolyses at similar rates substrates of the two different types and this observation has not been reported before. At the contrary, PGA was used in

Table 1
Conversion to solketal of diesters of type **a** (phenylacetates) and **b** (phenoxyacetates) with PVA and PGA at 48 h

Substrate type	Substitution pattern	PVA	PGA
a	*o*	33 (0.98)	92 (1.1)
	m	98 (1.3)	5 (0.01)
	p	18 (0.8)	–
b	*o*	98 (2)	15 (0.3)
	m	40 (1)	–
	p	22 (0.6)	–

Conditions described in Sections 2.4 and 2.5. Initial rates (20 min) in μmol/min are enclosed in parenthesis.

the enantioselective hydrolysis of the phenoxyacetate of an intermediate in the synthesis of *loracarbef* [8]. In our context, the *reversed substrate specificity* with PGA was not observed. In order to consider the biotransformation as synthetically useful, we carefully analysed the composition of the reaction mixture, in particular for the presence of the compound hydrolysed at only one of the two ester functionalities. No such compound was detected in any of the reaction mixtures. We consider therefore that the two bicarboxylic acids present in compounds of types **2a** and **3b** are candidates as potentially useful enzymatically removable alcohol-protecting groups.

The corresponding amides obtained from the latter and phenylethyl amine were prepared, but they resulted not to be good substrates of the two enzymes being hydrolysed at an exceedingly slow rate. In particular, due to the low water solubility of the amides, a biphasic system formed of water/methylene chloride was employed. In those conditions, the substrates hydrolysed from PVA had the same substitution pattern as those of the corresponding esters, but the maximum conversion observed was around 20%. Moreover, mixtures of compounds completely hydrolysed and partially hydrolysed were present. All together, the reaction could not be considered of any interest for the protection of primary amines.

4. Conclusions

meta-Bis-phenylacetic acid and *ortho*-bis-phenoxyacetic acid can be used as enzymatically removable alcohol-protecting groups in that the model esters of the two acids with solketal are readily and completely hydrolysed in mild conditions with the industrial enzyme PVA in an insoluble form (cross-linked cells). The bis-functional acids acting in the corresponding esters as linkers of two molecules of alcohol substrate, present advantages in the control of the enantiomeric excess of the intermediates in a multi-step synthesis, improving the enantiomeric purity of the final product.

Acknowledgements

We thank Dr. P.B. Poulsen for information concerning the origin of PVA which is at present not anymore in production at NOVO. This work was partially financed by CNR (Target project on biotechnology) and MURST, COFIN (Rome).

References

[1] P. D'Arrigo, L. Feliciotti, G. Pedrocchi-Fantoni, S. Servi, A. Strini, J. Org. Chem. 62 (1997) 6394.
[2] I. Fleming, S.K. Ghosh, J. Chem. Soc., Chem. Commun. 99 (1994) .
[3] V. Jungmann, H. Waldmann, Tetrahedron Lett. 39 (1998) 1139.
[4] A. Pundle, H.S. SivaRaman, Curr. Microbiol. 34 (1997) 144.
[5] D.A. Lowe, G. Romancik, R.P. Elander, Biotechnol. Lett. 8 (1986) 151.
[6] J.G. Shewale, V.K. Sudhakaran, Enzyme Microb. Technol. 20 (1997) 402.
[7] C. Fuganti, P. Grasselli, S. Servi, A. Lazzarini, P. Casati, J. Chem. Soc., Chem. Commun. 538 (1987) .
[8] M.J. Zmijeski, B.S. Briggs, A.R. Thompson, I.G. Wright, Tetrahedron Lett. 32 (1991) 1621.