Pergamon

Tetrahedron Letters 39 (1998) 5269–5272

TETRAHEDRON LETTERS

Acylase I catalysed hydrolysis of *para*-substituted (*S*)-phenylalanine derivatives from mixtures of the racemic *ortho*- and *para*-substituted isomers

Christopher J. Easton,[1]* Jason B. Harper

Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

Received 11 March 1998; accepted 11 May 1998

Abstract

para-Substituted (*S*)-phenylalanines may be obtained by treatment of the corresponding mixtures of *ortho*- and *para*-substituted *N*-acetyl-(*RS*)-phenylalanines with Acylase I from porcine kidney. The selectivity of the enzyme may be attributed to its evolution to digest peptide derivatives of (*S*)-phenylalanine and (*S*)-tyrosine.

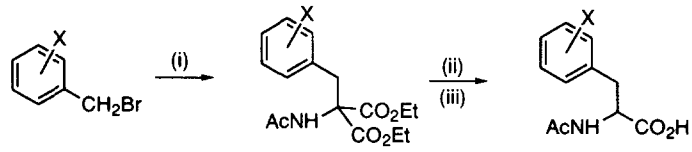
Keywords: Amino acids and derivatives; Enzymes and enzyme reactions; Regioselection; Stereoselection

Ring-substituted (*S*)-phenylalanines are important constituents of natural products, such as vancomycin [1,2]. Mixtures of *ortho*- and *para*-substituted phenylalanines may be synthesised, often as racemates, through electrophilic aromatic substitution of phenylalanine derivatives [3-5] and other aromatic starting materials [6,7]. Acylase I is a practical tool for resolution of a range of *N*-acetylamino acids [8,9]. We now report that it selectively digests *para*-substituted *N*-acetylphenylalanines over the corresponding *ortho*-substituted isomers, providing a convenient route for the preparation of *para*-substituted (*S*)-phenylalanines from the corresponding mixtures of *ortho*- and *para*-substituted *N*-acetyl-(*RS*)-phenylalanines.

For the present study, racemic samples of the *ortho*- and *para*-substituted *N*-acetylphenylalanines **1a,b-5a,b** were either prepared as illustrated in Scheme 1 [6] or by acetylation of commercially available substituted phenylalanines. Acylase I was purchased from Sigma Chemical Co. as a lyophilized powder with an activity of approximately 2×10^6 units g^{-1} (one unit will hydrolyse 1.0×10^{-6} moles of *N*-acetyl-(*S*)-methionine at pH 7.0 and 298 K in 1 h). Aqueous solutions containing racemic mixtures of regioisomeric pairs of the phenylalanine derivatives **1a,b-5a,b** (*ca.* 0.01 mol dm^{-3}) were treated with Acylase I (2.2×10^{-6} mol dm^{-3}), in 0.05 mol dm^{-3} pH 7.0 phosphate buffer, at 298 K for 2 h. The mixtures were then acidified, washed repeatedly with ethyl acetate and concentrated. The free amino acids present in the residues were treated with acetic anhydride in water and the

[1] email: easton@rsc.anu.edu.au; fax: 61 2 6249 5995

0040-4039/98/$19.00
PII: S0040-4039(98)00997-6



(i) $AcNHCH(CO_2Et)_2$, NaOEt/EtOH, 12 h; (ii) 6N HCl, Δ, 2 h; (iii) NEt_3, Ac_2O, H_2O, r.t., 3 h.

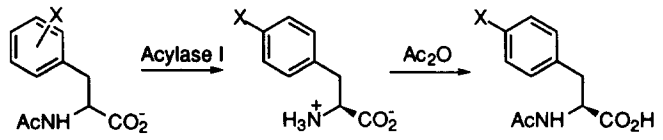
Scheme 1

product acetamides were isolated (Scheme 2, Table 1). They were shown to be enantiomerically pure, within the limits of detection, by conversion to the corresponding methyl esters using thionyl chloride pretreated methanol, followed by gas chromatographic analysis (GC) carried out on a Chirasil-Val® capillary column. The regioisomeric pairs **1a,b-5a,b** were distinguished using GC and ^1H NMR spectroscopy.

In four of the five cases investigated, Acylase I was specific for hydrolysis of the *para*-substituted *N*-acetyl-(*S*)-phenylalanine derivative. Control experiments established that separation of the regioisomers **1a,b-5a,b** is not an artefact of the isolation procedure. The systems studied included ring-substituted phenylalanines with hydrophobic, hydrophilic, electron-donating and electron-withdrawing groups, and indicate the broad tolerance of the enzyme for a ring substituent at the *para*-position. It was only in the case of the fluorophenylalanine derivatives **5a** and **5b** that any hydrolysis of the *ortho*-isomer was observed. This is presumably due to the fluoro substituent having comparable steric bulk to hydrogen [10] and, therefore, little effect on the interaction with the enzyme.

To determine the basis of the selectivity exhibited by the enzyme, kinetic experiments observing digestion by Acylase I were carried out on *N*-acetyl-(*S*)-phenylalanine and the derivatives (*S*)-**3a,b-5a,b**. Reactions were monitored by UV spectroscopy at 228 nm, the absorbance of the amide being hydrolysed. The rate constants of the enzyme catalysed reactions (k_{cat}) and the dissociation constants of the enzyme-substrate complexes (K_M) were calculated using Lineweaver-Burk analysis [11] (Table 2). Quite similar results were obtained using the curve-fitting programs KaleidaGraph® and MacCurvefit®. In the case of the methyl derivative **3b**, these values were determined using the racemate. Separate experiments gave values of k_{cat} and K_M for (*S*)-**5a,b** and *N*-acetyl-(*S*)-phenylalanine which were within experimental error whether the enantiomer or the racemate was used. This is consistent with published results [12] in which the (*R*)-enantiomers of other amino acids do not affect digestion of their (*S*)-antipodes.

In terms of their k_{cat}/K_M values, each of the *para*-substituted phenylalanine derivatives **3b-5b** is as good as or better than *N*-acetyl-(*S*)-phenylalanine as a substrate for Acylase I, and binds as or more readily to the enzyme (K_M), although the rate constant for reaction of the bound species (k_{cat}) is not necessarily higher. Although the *ortho*-methyl- and *ortho*-nitro-(*S*)-phenylalanine derivatives **3a** and **4a** were not digested by the enzyme, they each inhibit catalysis of the reaction of *N*-acetyl-(*S*)-phenylalanine. With the methylphenylalanine derivative **3a**, the dissociation constant of the enzyme-inhibitor complex, K_i, was measured as $(1.5 \pm 0.3) \times 10^{-3}$ mol dm^{-3}. Thus it seems likely that *ortho*-substituted phenylalanine derivatives bind to the enzyme, but not with the correct orientation for reaction.



Scheme 2

Table 1
Results of digestion of racemic mixtures of the *N*-acetylphenylalanines **1a,b-5a,b** by Acylase I

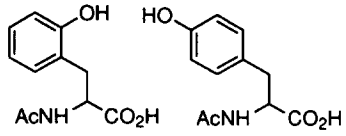
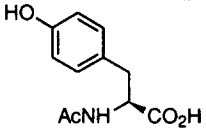
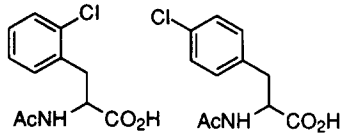
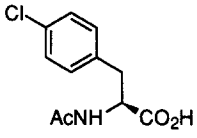
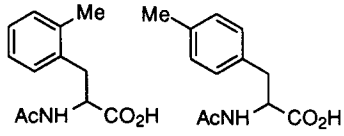
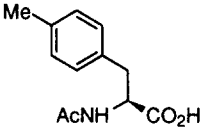
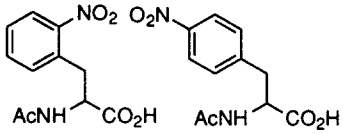
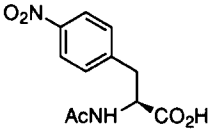
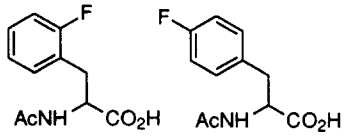
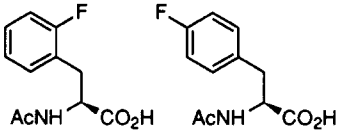
Starting Materials (ratio 1:1)	Products	Yield
 **1a** **1b**	 (*S*)-**1b**	73%
 **2a** **2b**	 (*S*)-**2b**	71%
 **3a** **3b**	 (*S*)-**3b**	72%
 **4a** **4b**	 (*S*)-**4b**	83%
 **5a** **5b**	 (*S*)-**5a** (*S*)-**5b**	29%, 58%

Table 2
Rate constants for the reaction of bound substrates and dissociation constants for the Michaelis complexes of *N*-acetylphenylalanines with Acylase I

N-Acetylphenylalanine	k_{cat} (s^{-1})	K_M (x 10^3 mol dm^{-3})	k_{cat}/K_M (x 10^{-3} mol^{-1} dm^3 s^{-1})
(*S*)-**3a**		no digestion detected	
(*S*)-**3b**	2.7 ± 0.2	0.32 ± 0.04	8 ± 2
(*S*)-**4a**		no digestion detected	
(*S*)-**4b**	15 ± 1	0.50 ± 0.07	30 ± 6
(*S*)-**5a**	7.3 ± 0.4	1.0 ± 0.1	7 ± 1
(*S*)-**5b**	66 ± 1	3.3 ± 0.1	20 ± 1
N-acetyl-(*S*)-phenylalanine	24 ± 4	3.9 ± 0.8	6 ± 2

The natural substrates of Acylase I are phenylalanine and tyrosine derivatives. On this basis it seems likely that the enzyme has evolved to tolerate *para*-substituted phenylalanine derivatives, whereas the *ortho*-isomers are not processed because natural substrates of this type are not prevalent. In any event, Acylase I effectively discriminates between *ortho*- and *para*-substituted (*S*)-phenylalanine derivatives.

Acknowledgment

JBH gratefully acknowledges the receipt of the Shell Australia Postgraduate Scholarship.

References

[1] Williams DH. Acc. Chem. Res. 1984;17:364-369.
[2] Rama Rao AV, Gurjar MK, Reddy KL, Rao AS. Chem. Rev. 1995;95:2135-2167.
[3] Bergel F, Stock JA. J. Chem. Soc. 1954:2409-2417.
[4] Shimidzu T, Iyoda T, Kanda N. J. Chem. Soc., Chem. Commun. 1981:1206-1207.
[5] Coenen HH, Bodsch W, Takahashi K, Hossman A, Stoecklin G. Nuklearmedizin, Suppl. 1986;22:600-602.
[6] Vasil'eva MN, Shkodinskaya EN, Berlin AY. Zh. Obsch. Khim., 1961;31:1027-1033.
[7] Cleland GH. J. Org. Chem. 1969;34:744-747.
[8] Chenault HK, Dahmer J, Whitesides GM. J. Am. Chem. Soc. 1989;111:6354-6364.
[9] Bommarius AS, Drauz K, Günther K, Gnaup K, Schwarm M. Tetrahedron Asymm. 1997;8:3197-3200.
[10] Clark JH, Wails D, Bastock TW. Aromatic fluorination. Boca Raton: CRC Press, 1996.
[11] Lineweaver H, Burk D. J. Am. Chem. Soc. 1934;56:658-666.
[12] Birnbaum SN, Levinton L, Kingsley RB, Greenstein JP. J. Biol. Chem. 1952;194:455-470.