An Esterase with β-Lactamase Activity

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Porcine liver esterase selectively catalyses the hydrolysis of the β-lactam of the methyl ester of benzylpenicillin with a k_{cat}/K_m of 675 $dm^3\ mol^{-1}\ s^{-1}$ and without hydrolysing the methyl ester function.

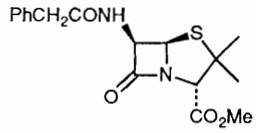
The fidelity of enzymes to their so-called 'natural' substrates and type of reaction catalysed is part of the folk-lore of enzymology. It has, however, been stated that enzymes can catalyse different reactions so long as they involve similar electron density changes.[1] Peptidases are known to catalyse the hydrolysis of not only 'natural' peptide substrates but also chemically reactive esters.[2] Esterases, on the other hand, are thought to be able to catalyse only the hydrolysis of esters. The degree of selectivity for esterases is important because of their ubiquity and the increasing use of pro-drugs masked as esters. The conversion of some cephalosporins, a member of the β-lactam family of antibiotics,[3] into esters confers them with inhibitory properties of human leukocyte elastase.[4]

Porcine liver esterase (PLE, E.C. 3.1.1.1.) is widely used in asymmetric synthesis[5,6] and because of our interest in using it to synthesise β-lactams we had cause to examine its reactivity with penicillin derivatives. Surprisingly, PLE catalyses the hydrolysis of the methyl ester of benzylpenicillin **1** to the methyl ester of (3*S*,5*R*,6*R*)-benzylpenicilloate **2**. The enzyme catalyses the hydrolysis of the β-lactam ring more efficiently than that of the methyl ester.

The penicillin ester **1**, the ring opened derivative **2**, benzylpenicillin **3** and benzylpenicilloate **4** are easily identifiable by HPLC using a Lichrosorb C18 reversed phase column and an eluent system consisting of acetonitrile–0.1% trifluoroacetic acid (36 : 65) solution. The retention times are as follows: **1** 10.3 min; **2** 5.9 min; **3** 5.5 min; **4** 3.4 min.

The hydrolysis of **1** catalysed by *Bacillus cereus* β-lactamase gives the same product **2** which co-elutes with the product from the esterase catalysed hydrolysis. No epimerisation at C-6, C-5 or C-3 occurs during the esterase catalysed hydrolysis. The NMR spectrum of the hydrolysis product is identical to that of **2** and shows the intact methyl ester at δ 3.71 and the ring opened 5-H and 6-H protons at δ 5.10 and 4.76 respectively with $J_{5,6}$ = 4 Hz.

The second-order rate constant k_{cat}/K_m for the PLE catalysed hydrolysis of the β-lactam is 675 $dm^3\ mol^{-1}\ s^{-1}$ at pH 7.80 and 25 °C.



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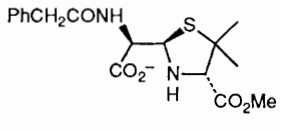

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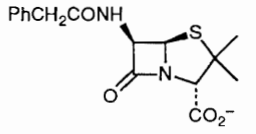

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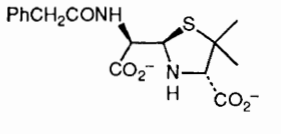

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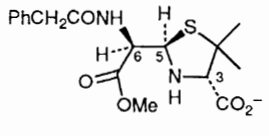

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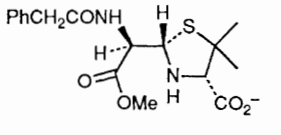

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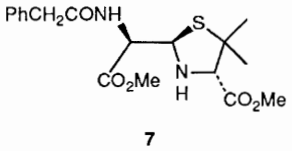

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Enantioselectivity is observed in the PLE catalysed hydrolysis of the methyl ester of benzyl penicilloate with respect to the chiral centre at carbon-3 of the carboxylic acid ester. The methyl ester of (3*S*,5*R*,6*R*)-benzylpenicilloate **5** is hydrolysed by PLE while the (3*S*,5*S*,6*R*)-epimer **6** is not a substrate.

The pure methyl ester of (3*S*,5*R*,6*R*)-benzylpenicilloate **5** is readily prepared from the methanolysis of benzylpenicillin and a diastereoisomeric mixture of this and the (3*S*,5*S*,6*R*) isomer **6** is obtained from this by leaving an aqueous solution of **5** at pH 4.0 for two days.

The two esters are easily distinguished by their NMR spectra [(3*S*,5*R*,6*R*)-isomer: δ 1.22 (s, 2-α-Me), 1.52 (s, 2-β-Me); (3*S*,5*S*,6*R*)-isomer: δ 0.93 (s, 2-α-Me), 1.64 (s, 2-β-Me)] and from the hydrolysis products by HPLC eluting with acetonitrile–phosphoric acid (0.1 mol dm^{-3}) (30:70) with sodium hexanesulphonate (4.8×10^{-3} mol dm^{-3}) on a 5μ reversed phase column of polystyrene–divinylbenzene the retention times of the 5*R* and 5*S* esters are 9.5 and 9.1 min, respectively.

No hydrolysis of the esters (10.3 mg in 10 cm^3, 2.65×10^{-3} mol dm^{-3}) occurs at pH 8.0, tris buffer at 25 °C, 3 h. In the presence of 4×10^{-6} mol dm^{-3} porcine liver esterase, the methyl ester of the (3*S*,5*R*,6*R*]-epimer is hydrolysed with a k_{cat}/K_m of 30 dm^3 mol^{-1} s^{-1} to give (3*S*,5*R*,6*R*)-benzylpenicilloic acid **4** (retention time 4.6 min).

The rate of production of the acid **4** equals the rate of disappearance of the ester **5**. The hydrolysis of an epimeric mixture of the 5*R* and 5*S* esters using PLE selectively hydrolyses the 5*R* epimer whilst the 5*S* ester remains unhydrolysed.

It is noted that the PLE catalysed hydrolysis presumably proceeds *via* the intermediate formation of a penicilloyl enzyme, analogous to that formed during the reaction of penicillin with β-lactamase and D,D-peptidase enzymes.[3] However, there was no evidence that benzylpenicillin was formed during the enzyme catalysed hydrolysis of either **5** or the dimethyl ester **7**, which, similar to **1** selectively yielded the monoester **2**.

If the β-lactam carbonyl of benzylpenicillin is placed adjacent to the active serine, the thiazolidine residue can occupy the adjacent large hydrophobic pocket in the model of PLE suggested by Jones *et al.*[5] Similarly the diesters can be made to fit this model although in both cases a large fraction of the molecule lies outside the proposed cavities.

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