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Porcine Pancreatic Lipase Catalyzed Enantioselective Hydrolysis of Esters of N-Protected Unusual Amino Acids

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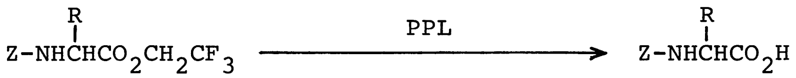
Porcine pancreatic lipase catalyzed the highly enantioselective hydrolysis of a kind of α-substituted carboxylic esters, i.e., the 2,2,2-trifluoroethyl esters of the N-benzyloxycarbonyl derivatives of unusual amino acids.

During the last several years, microbial lipases from a variety of sources have become obtainable commercially and inexpensively as well, and the number of their applications to stereoselective[1)] or regioselective[2)] organic syntheses has been increasing. Apart from these microbial enzymes, a lipase from porcine pancreas (PPL) has been known for a long time.[3)] Since this enzyme is the most inexpensive among lipases, it is of practical value to utilize its enzymatic activity more efficiently in organic syntheses. PPL has been used with success mainly for the enantioselective hydrolysis of esters of racemic alcohols[4)] and meso-diols.[5)] On the other hand, only a few reports have dealt with esters of carboxylic acids: esters of meso-dicarboxylic acids,[6)] racemic dicarboxylic acids,[7)] and a β-substituted carboxylic acid (3,4-epoxybutanoic acid)[8)] have been hydrolyzed enantioselectively by this enzyme. By contrast, however, a careful examination of the scarce literature data[9)] tells us that it displayed an extremely low enantioselectivity toward esters of α-substituted carboxylic acids, which constitute a class of important chiral building blocks. We now report that esters of the N-benzyloxycarbonyl (Z) derivatives of unusual amino acids, which belong to chiral α-substituted carboxylic esters, can be hydrolyzed by PPL[10)] with high enantioselectivities.

The easily removable Z group was chosen for the N-protection of amino acids.[11)] The methyl esters, for example, of the Z-amino acids were not hydrolyzed by PPL, and accordingly active esters such as 2,2,2-trifluoroethyl, 2-chloroethyl, 2,2,2-trichloroethyl, or cyanomethyl esters were tested; except the last one, they generally displayed no significant difference in the reaction rate and enantioselectivity. Table 1 shows the results on the hydrolysis of the 2,2,2-trifluoroethyl esters (1) of the Z derivatives of a number of unusual amino acids.[12)] The results with some proteinic amino acids are also included for comparison. The enzymatic hydrolyses were carried out in phosphate buffer (pH 7.0, 0.2 M) at 25 °C. After the desired degree of conversion (ca. 40%), the liberated Z-amino acids (Z-AA) (2) were isolated and their optical purities were determined by HPLC after conversion to the diastereomeric peptides Z-AA-Gly(or Sar)-L-Phe-OMe[13),14)] and/or to free amino acids.[15)]

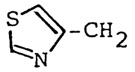


$$\text{Z-NH}\overset{\text{R}}{\overset{|}{\text{C}}}\text{HCO}_2\text{CH}_2\text{CF}_3 \xrightarrow{\text{PPL}} \text{Z-NH}\overset{\text{R}}{\overset{|}{\text{C}}}\text{HCO}_2\text{H}$$

1 2

The esters 1 of unusual amino acids with a variety of side-chain structures were hydrolyzed by PPL with high enantioselectivities. The valine derivative (1, R = i-C_3H_7) with a β-branching was not a substrate for this enzyme, while the esters with a γ- or δ-branching (1, R = i-C_4H_9 or i-C_5H_{11}) were hydrolyzed within a reasonable time. In all the cases, the preferential hydrolysis of the L-enantiomers[16)] was confirmed by direct comparison with authentic samples, if available, or suggested from the regularity of elution order of the peptide diastereomers[14)] or the amino acid enantiomers,[15)] on HPLC. From the results in Table 1, it is worth noting that the enantioselectivity in the PPL-catalyzed hydrolysis depends largely on the length of the side chain (R) of an amino acid. For the amino acids with aliphatic side chains, high enantioselectivities (87 - 98 % e.e.) were obtained with alkyl groups of 2 to 5 carbon atoms, while the e.e.-

Table 1. PPL-catalyzed Hydrolysis of 1[a)]

R	% Convn.	% e.e.[b)]
CH_3	39	21
C_2H_5	40	97
n-C_3H_7	39	98
n-C_4H_9	38	92
i-C_4H_9	40	98
n-C_5H_{11}	40	87
i-C_5H_{11}	40	95
n-C_6H_{13}	33	61
n-C_7H_{15}	21	52
CH_2=$CHCH_2$	38	93
$CH_3S(CH_2)_2$	40	90
$C_2H_5S(CH_2)_2$	42	87
C_6H_5	44	97
$C_6H_5CH_2$	40	>99
2-$FC_6H_4CH_2$	40	97
3-$FC_6H_4CH_2$	40	95
4-$FC_6H_4CH_2$	40	90
4-$ClC_6H_4CH_2$	28	94
$C_6H_5CH_2CH_2$	30	71
$C_6H_5CH_2(CH_2)_2$	17	36
	44	89

a) Hydrolyses were carried out with 1 mmol of 1 in the presence of 300 mg of PPL in 2 ml of 0.2 M phosphate buffer (pH 7.0) at 25 °C. During the reaction, 0.5 M NaOH was added to maintain the pH constant. The reaction times were 18 - 47 h for the listed conversions with the following exceptions: R = CH_3, 170 h; R = n-C_6H_{13}, 10 d; R = n-C_7H_{15}, 18 d; R = 4-$FC_6H_4CH_2$, 117 h; R = 4-$ClC_6H_4CH_2$, 12 d; R = $C_6H_5CH_2(CH_2)_2$, 24 d. b) Enantiomeric excess of 2.

values become much lower with those of 6 or more carbon atoms and also with that of one carbon atom. This is also the case with the aromatic amino acids, if an aromatic ring is supposed to correspond to a straight chain of 4 carbon atoms. This result offers a valuable piece of information to establish the structural requirements for a PPL-catalyzed highly enantioselective hydrolysis of esters.[17)]

To our knowledge, this is the first successful example of PPL-catalyzed enantioselective hydrolysis of chiral α-substituted monocarboxylic esters, and the hydrolyses of esters of other carboxylic acids of this group are now under investigation.

References

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3) R. Verger, "Lipases," ed by B. Borgström and H. L. Brockman, Elsevier, Amsterdam (1984), p. 83.

4) For a recent example, see: B. A. Marples and M. Rogers-Evans, Tetrahedron Lett., 30, 261 (1989).

5) For a recent example, see: G. Guanti, L. Banfi, and E. Narisano, Tetrahedron Lett., 30, 2697 (1989).

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9) G. Fülling and C. J. Sih, J. Am. Chem. Soc., 109, 2845 (1987); R. Dernoncour and R. Azerad, Tetrahedron Lett., 28, 4661 (1987).

10) Lipase Type II from porcine pancreas (steapsin), supplied by Sigma Chemical Co., U.S.A.

11) R. Geiger and W. König, "The Peptides," ed by E. Gross and J. Meienhofer, Academic Press, New York (1981), Vol. 3, Chap. 1; the Z group was found to be preferable to other N-protecting groups from the results on the microbial lipase catalyzed hydrolyses (Cf. Ref. 12).

12) About half of the unusual amino acids listed in Table 1 were used in the microbial lipase catalyzed hydrolysis; T. Miyazawa, T. Takitani, S. Ueji, T. Yamada, and S. Kuwata, J. Chem. Soc., Chem. Commun., 1988, 1214.

13) Sar: sarcosine (N-methylglycine).

14) On an ODS column; see Ref. 12. In general, the L-L-diastereomer is eluted first.

15) On a Crownpak CR(+) column (4 mm I.D. x 150 mm) (Daicel Chemical Industry, Ltd., Japan) under the following conditions: mobile phase, $HClO_4$ aq. (pH 1.5 - 3.0); flow rate, 0.4 - 0.8 ml/min; column temperature, 25 °C; detection, 200 nm. For usual amino acids, the D-isomer is generally eluted first.

16) In some cases, the unhydrolyzed esters were also isolated and their optical purities were determined after saponification and the same derivatization as in the case of Z-AA: for example, 86% e.e. (D) at 48% conversion for the ester 1, R = $n\text{-}C_3H_7$.

17) In the PPL-catalyzed hydrolysis of esters of epoxy alcohols, it has been reported that the enantioselectivity depends on the length of the alkyl group of the acid component of the ester: W. E. Ladner and G. M. Whitesides, J. Am. Chem. Soc., 106, 7205 (1984).

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