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Letter

Ferulic acid esterase from *Humicola Insolens* catalyzes enantioselective transesterification of secondary alcohols

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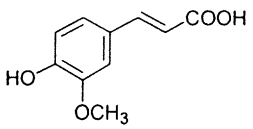
Abstract

Ferulic acid esterase (FAE) from *Humicola insolens* was found to catalyze transesterifications of secondary alcohols with high enantioselectivity. In all cases the enzyme showed *R* enantiopreference.

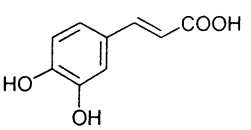
Keywords: Ferulic acid; Esterase; Transesterification; Enantioselectivity; Secondary alcohols

Ferulic acid esterases (FAE) [1] are enzymes which hydrolyze the esters of hydroxycinnamic acids [2] that are components of plant cell walls. These esters enhance the mechanical strength of the cell wall [3] and limit cell wall growth [4] by cross-linking hemicellulose and pectin [5,6]. These esterases are secreted by many organisms, such as bacteria [7,8] and fungi [9,10], their role being to facilitate the breakdown of the plant cell wall either to enter the plant cell wall or to directly utilize its material as a nutritional resource. These enzymes are important components of microbial xylanolytic enzyme systems and are required for the efficient degradation of cell-wall polymers [11,12].

In addition to their fundamental biological importance feruloyl esterases have many potential industrial and medicinal applications. Increasing interest in feruloyl esterases revolves around their potential applications in the food and flavour industries, as ferulic acid and other hydroxycinnamic acids could be used as flavour precursors [13]. Furthermore, ferulic acid shows photoprotective [14], antioxidant [15], antitumor [16,17] and hypotensive [18] activity. Most of the research done to date involves the isolation, purification and characterization of feruloyl esterases derived from a wide range of microorganisms [7–10], as well as the enzymatic release of the products from cell-walls degradation [3–6]. In all cases the activities of these enzymes have been determined by the release of free ferulic acid or other hydroxycinnamic acids. A few studies on substrate specificity and on the kinetics towards a range of methyl phenylalkanoates have been used to probe the active site of the enzyme [2,19].



ferulic acid



3,4-dihydroxy-cinnamic acid

Recently, the crystal structure of feruloyl esterase module of xylanase 10B [20] and xylanase Z [21] from *Clostridium thermocellum* were reported. The primary structure analysis of FAEs has shown that they display α/β hydrolase fold, with an Asp/His/Ser catalytic

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Table 1

Entry	Substrate[a]	Conversion (%)	ee (%)	t (d)	Configuration	E[b]
1	1-(4-Pyrinidinyl)-1-ethanol	50	>98	2	R[c]	>100
2	1-(1,1′-Biphenyl)-4-yl-1-ethanol	17	>98	2	–	>100
3	1-Phenyl-1-ethanol	23	92	3	R[d]	32
4	1-Phenyl-1-propanol	25	93	3	R[e]	37
5	1-(4-Methoxyphenyl)-1-ethanol	18	88	3	R[f]	19
6	1-(4-Isopropylphenyl)-1-ethanol	7	93	7	–	30
7	1-(3-Methylphenyl)-1-ethanol	52	91	5	R[g]	>100

[a] Alcohols 3 and 4 were commercially available. All the other alcohols were prepared by Grignard addition of CH_3MgI to the corresponding aldehydes.

[b] [28].

[c] The configuration of the ester was determined by the optical rotation of the isolated product after 50% conversion. Measured $[a]_D^{25} = +81 \pm 1$ (c 1, $CHCl_3$) and literature $[a]_D^{25} = +81$ (c 1, $CHCl_3$) [25].

[d] By GC analysis with a chiral capillary column of the remaining alcohol and standard *S*-(−)-1-phenyl-1-ethanol.

[e] By the optical rotation of the isolated product after 50% conversion. Measured $[a]_D^{25} = +47 \pm 1$ (c 1, $CHCl_3$) and literature $[a]_D^{25} = +49$ (c 1, $CHCl_3$) [26].

[f] By the optical rotation of the remaining alcohol after 35% conversion. Measured $[a]_D^{25} = +-15$ (c 1, $CHCl_3$), ee% 30 and literature $[a]_D^{25} = +52$ (c 1, $CHCl_3$), ee % 87 (*R*)-alcohol, [26].

[g] By comparison of GC analysis of the produced ester and the enzymatic hydrolysis of racemic ester of alcohol 7 with PFL [27].

triad at their active site [20–22]. Despite the fact that feruloyl esterases and lipases bear the same catalytic triad of residues in their active site they do not show lipase activity. Recently, the synthesis of pentylferulate was achieved by using a water-in-oil microemulsion system containing a feruloyl esterase from *Aspergillus niger* [23]. Nevertheless, the potential of feruloyl esterases for the transesterification of alcohols has never been reported.

In this work, we have examined for the first time the reactivity of a crude feruloyl esterase preparation from *Humicola Insolens* towards substrates that bear no structural similarity to the natural substrates for this enzyme. Our preliminary results show that this crude enzyme preparation can successively catalyze transesterifications of secondary alcohols with high enantioselectivities. These are the first reported stereoselective reactions catalyzed by this enzyme, and may eventually lead to the use of this enzyme as a potent catalyst in stereoselective organic synthesis.

In a typical run, 200 mg of the enzyme preparation[1] was placed in a vial to which was added 0.2 mmol of the substrate and a 30-fold excess of vinyl acetate, which was used as the activated acyl donor [24]. The resulting suspension was shaken at 45 °C. Samples were collected periodically and were analyzed by gas chromatography. The enantioselectivity in each reaction was determined by analyzing the optical purities of the unreacted alcohol and the produced ester by using a 30 m chiral capillary column (HP-5 cross-linked 5% phenyl-methyl silicone). The results are summarized in Table 1.

From the results in Table 1, by looking at the reaction times, it is obvious that the enzyme shows relatively similar reactivity towards the studied alcohols, except for alcohol 6 where the reaction is much slower. In all cases FAE showed high enantioselectivity, up to 98% ee, with *R* enantiopreference, as determined from the measured optical rotations. It should be noted here that in the cases of 1-(4-pyridinyl)-1-ethanol, 1, and 1-(3-methylphenyl)-1-ethanol, 7, when the conversion was extended to 60%, the unreacted alcohol was almost enantiopure (>99% ee) having the *S* configuration. When the above catalytic reactions were run at room temperature, the reaction times were longer than those at 45 °C and the enantioselectivities observed were slightly higher.

In summary, our preliminary results have shown that feruloyl esterase from *Humicola Insolens*, which

[1] The enzyme preparation (Pentopan 500 BG) was given by Novo Nordisk as a commercial culture supernatant from a strain of *Humicola Insolens*. The FAE activity of the enzyme preparation was 0.435 U/gr.

naturally degrades a wide range of ferulic acid esters, can successively catalyze the stereoselective transesterification of secondary alcohols that bear no structural similarity to the natural substrates of this enzyme. These reactions proceed with good to excellent enantioselectivity analogous to that of lipases [25–27]. This unique behavior of the feruloyl esterase has never been reported before. Further studies are currently under way in our laboratories in order to gain a better insight on substrate specificity and to explore the usefulness of this enzyme as a potent catalyst for the enantioselective synthesis of various alcohols and esters with interesting practical applications.

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References

[1] L.A. Deobald, D.L. Crawford, Appl. Microbiol. Biotechnol. 26 (1987) 158.
[2] P.A. Kroon, C.B. Faulds, C. Brezillon, G. Williamson, Eur. J. Biochem. 248 (1997) 245.
[3] K. Iiyama, T.B. Lam, B. Stone, Plant Physiol. 104 (1994) 315.
[4] G. Berg, T. Cajal, G.L. Butterfoss, R.L. Grey, M.A. Alsina, B.Z. Yu, M.K. Jain, Biochemistry 37 (1998) 6615.
[5] T. Ishi, Plant Science 127 (1997) 111.
[6] G. Williamson, C.B. Faulds, P.A. Kroon, Biochem. Soc. Trans. 26 (1998) 95.
[7] L.M.A. Ferreira, T.M. Wodd, G. Williamson, C.B. Faulds, G.P. Hazlewood, H.J. Gilbert, Biochem. J. 294 (1993) 349.
[8] C.B. Faulds, G. Williamson, J. Gen. Microb. 137 (1991) 2339.
[9] M. Tenkanen, J. Schuseil, J. Puls, K. Poutanen, J. Biotechnol. 18 (1991) 69.
[10] P.A. Kroon, C.B. Faulds, G. Williamson, Biotechnol. Appl. Biochem. 23 (1996) 255.
[11] N. Kulkarni, A. Shendye, M. Rao, FEMS Microbiol. Rev. 23 (1999) 411.
[12] B.L. Garcia, A.S. Ball, J. Rodriguez, M.I. Rerez-Leblic, M.E. Arias, J.L. Copa-Patino, FEMS Microbiol. Lett. 158 (1998) 95.
[13] M.J. Gasson, N.J. Walton, J. Biol. Chem. 273 (1998) 4163.
[14] A. Saija, A. Tomaino, D. Tromdetta, A. De Pasquale, N. Uccella, T. Barbuzzi, D. Paolino, F. Bonita, Int. J. Pharm. 199 (2000) 39.
[15] H. Kikyzaki, M. Hisamoto, K. Hirose, K. Akiyama, H. Tanigughi, J. Agric. Food Chem. 50 (2002) 2161.
[16] M.J. Margovich, A. Jimenez, K. McKee, V.E. Steele, M. Velasco, J. Woods, R. Price, K. Gray, G.J. Kellof, Carcinogenesis 21 (2000) 1149.
[17] H. Mori, K. Kawabata, N. Yoshimi, T. Tanaka, T. Murakami, T. Okada, H. Muray, Anticancer Res. 19 (1999) 3775.
[18] A. Susuki, D. Kagawa, A. Futji, R. Ochiai, I. Tokimitsu, I. Saito, Am. J. Hypertens. 15 (2002) 351.
[19] G. Williamson, C.B. Faulds, P.A. Kroon, Biochem. Soc. Trans. 26 (1998) 205.
[20] J.A.M. Prates, N. Tarbouriech, S.J. Charnock, C.M.G.A. Fontes, L.M.A. Ferreira, G.J. Davies, Stucture 9 (2001) 1183.
[21] F.D. Schubot, I.A. Kataeva, D.L. Blum, A.K. Shah, L.G. Ljungdahl, J.P. Rose, B.-C. Wang, Biochemistry 40 (2001) 12524.
[22] P.A. Kroon, G. Williamson, N.M. Fish, D.B. Archer, N.J. Belshaw, Eur. J. Biochem. 267 (2000) 6740.
[23] S. Giuliani, C. Piana, L. Setti, A. Hochkoeppler, P.G. Pifferi, G. Williamson, C.B. Faulds, Biotechnol. Lett. 23 (2001) 325.
[24] H.-F. Wang, J.J. Lalonde, M. Momongan, D.E. Bergbreiter, C.-H. Wong, J. Am. Chem. Soc. 110 (1988) 7200.
[25] R. Seemayer, M.P. Schneider, Tetrahedon: Asymmetry 3 (1992) 827.
[26] A.J.M. Janssen, A.J.H. Klunder, B. Zwanenburg, Tetrahedron 47 (1991) 7645.
[27] K. Laumen, M.P. Schneider, J. Chem. Soc., Chem. Commun. 1988, p. 598.
[28] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.