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Letter to the Editor

Commercial enzyme preparations catalyse feruloylation of glycosides

Abstract

Commercial enzyme preparations from *Humicola insolens*, *Thermomyces lanuginosus* and *Aspergillus niger* were tested for transesterifications of ferulic acid from its active esters to various glycosides at their primary hydroxyl group. Use of Lipolase 100T (from *T. lanuginosus)* was optimized for these syntheses in preparative scale.

Keywords: Feruloylated glycosides; Transesterification; *Humicola insolens*; *Thermomyces lanuginosus*; *Aspergillus niger*

Feruloylated carbohydrates are common components of plant cell walls as parts of lignin–carbohydrate complexes [1–3]. They enhance the mechanical strength of cell walls through a cross-linking of polysaccharide chains by dimerisation of hydroxycinnamic acids [4]. Due to the presence of ferulate moiety exhibiting a powerful antioxidant, photoprotective and antitumor activity, feruloylated carbohydrates and their fragments may potentially find use in food, cosmetic, pharmaceutical and material industries. Environmentally friendly preparation of defined structures of feruloylated monosaccharides and glycosides is therefore a challenge for researchers active on the field of biocatalysis.

Feruloyl esterases [E.C. 3.1.1.73] (FAEs) (also known as cinnamoyl esterases) represent a group of carboxylic acid esterases able to hydrolyse an ester bond between hydroxycinnamic acid and sugars present in plant cell walls [3,5]. In recent years the number of reports about microbial FAEs activities has increased and FAEs have been classified into four types based on their specificity [5]. Utilisation of different commercial enzyme preparations exhibiting feruloyl esterase side-activity to release ferulic acid or its dimer forms from plant cell walls is a developing tendency [6–8]. Although some reports describe the enzymatic synthesis of alkyl [9–11] and glyceride [12] ferulates, there exist few papers concerning enzymatic preparation of feruloylated carbohydrates. The commercial lipase Novozym 435 was used for synthesis of feruloylated flavonoid glycosides [13], while feruloylated L-arabinose was obtained by use of feruloyl esterase from *Sporotrichum thermophile* [14,15]. Conversion of products did not exceed 40% yield. Noteworthy, some feruloyl transferases occurring in plant cells have been used for feruloylation of flavonoids [16] or arabinoxylan-trisaccharide [17].

To overcome disadvantages of chemical [18] or chemoenzymatic [19] regioselective synthesis of feruloylated carbohydrates, our attention was paid to alternative direct enzymatic ways with no requirement for elaborate protection/deprotection strategies. In this work, we have looked for cheap possibilities, realised in preparative scale and good yields. Four crude commercial enzyme preparations were tested for presence of feruloyl esterase activity. The enzyme activity was determined by spectrophotometric assay by measuring the released 4-nitrophenol from 4-nitrophenyl ferulate [20] according to a previously published method [21]. As indicated in Table 1, the preparations comprising the highest levels of feruloyl esterase are all coming from *Humicola insolens, Thermomyces lanuginosus* (formerly *H. lanuginosa*) or *Aspergillus niger*.

Enzyme preparations from Table 1 have been tested in transferuloylations in various organic solvents. Different types of glycosides as acceptors have been selected: methyl α-L-arabinofuranoside (**1**) as a type of alkyl pentofuranoside, methyl α-D-glucopyranoside (**2**), esculine (**3**) and *n*-pentyl β-D-galactopyranoside (**4**) as representatives of alkyl and aryl hexopyranosides. Active vinyl [13] and 2,2,2-trifluoroethyl [20] esters of ferulic acid (VnFe and TfeFe) have been used as feruloyl donors.

In a typical screening run, glycoside (1 mmol), vinyl or 2,2,2-trifluoroethyl ferulate (2 mmol, 2 equiv.) and enzyme preparation (400 mg or 400 μl) was added to 2 ml of organic solvent at room temperature. The resulting suspensions were shaken at 37 °C for several hours (Table 2). Samples were periodically analysed by TLC on silica-gel 60 F_{254} (Merck) in solvent system comprising chloroform/methanol (4:1 (v/v)); R_f were 0.70 for VnFe and TfeFe, 0.25 for **1**, 0.09 for **2**, 0.07 for **3**, 0.17 for **4**, 0.42 for **5**, 0.26 for **6**, 0.18 for **7** and 0.35 for **8**.

Results estimated from intensity of spots on TLC and summarised in Table 2 indicate that use of liquid enzyme

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Table 1
Comparison of feruloyl esterase activity in investigated commercial enzyme preparations in aqueous buffer

Enzyme preparation	Type of enzyme	Origin	Company	Specific activity (U/g)[a] (U/ml)[b]
Lipase A	Lipase	*Aspergillus niger*	Amano	55.5[a]
Pentopan 500 BG	Xylanase	*Thermomyces lanuginosus*	Novozymes	39.7[a]
Ultraflo L	Hemicellulase	*Humicola insolens*	Novozymes	12.0[b]
Lipolase 100L	Lipase	*T. lanuginosus*	Novozymes	8.0[b]
Lipolase 100T	Lipase	*T. lanuginosus*	Novozymes	–[c]

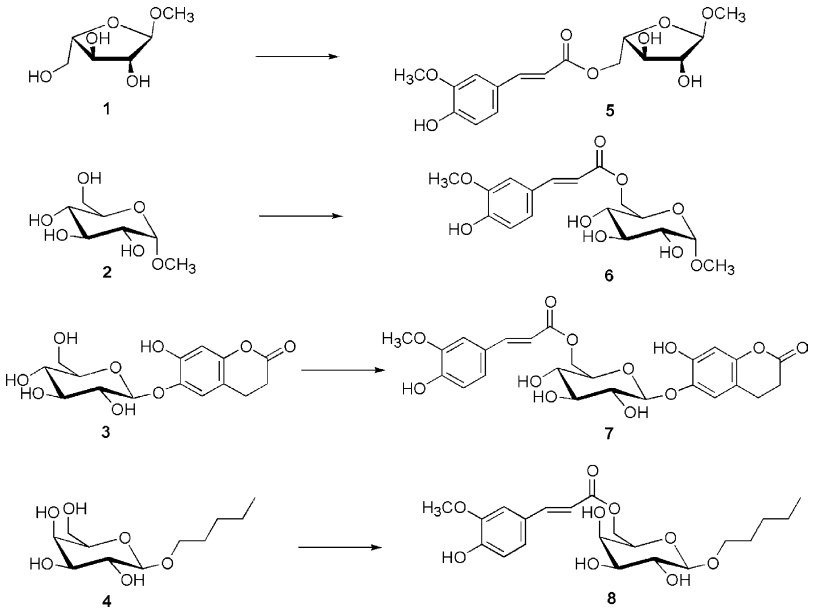
[a] Solid enzyme, activity in U/mg.
[b] Liquid enzyme, activity in U/ml.
[c] The enzyme is probably linked to support, thus it was impossible to dissolve it in buffer.

preparations or conditions including some amount of water are undesirable (entries 14, 15, 19) since active esters of ferulic acid have been preferentially hydrolysed. Better results have been acquired with solid immobilised enzyme preparations. There was however observed some effect of structure and solubility of glycosides as well as nature of organic environment on the rate and yield of feruloylation. Although lipase A satisfactory catalysed feruloylation of L-arabinofuranoside **1** (entry 4), only moderate yield of feruloylation was found in the case of D-pentopyranoside **4** (entry 30) while feruloylation on **2** did not proceed (entry 16). No feruloylation had occurred in more hydrophobic solvents like toluene or diisopropyl ether (entries 1, 7, 8, 20), probably due to insolubility of the acceptor glycosides. Glycoside **4** had the best solubility in used organic solvents and small amount of feruloylated product was observed even in toluene (entry 25). Pentopan 500BG did not work well in tert-butanol (entries 18, 29), but very good in chloroform (entries 5, 6, 26), acetonitrile (entries 17, 28) or methyl isobutyl ketone (entry 27). Use of *tert*-butanol as solvent under catalysis of Lipolase 100T offered only moderate yields (entries 13,

Table 2
Screening of conditions for feruloylation of selected glycosides

Entry	Saccharide	Activated aromatic ester	Enzyme preparation	Solvent	Time (h)	Degree of acylation[a]
1	**1**	TfeFe	Lipolase 100T	Toluene	46	–
2	**1**	VnFe	Lipolase 100T	Chloroform	72	++
3	**1**	VnFe	Lipolase 100T	Acetonitrile	48	+++
4	**1**	VnFe	Lipase A	Acetonitrile	70	++
5	**2**	TfeFe	Pentopan 500 BG	Chloroform	72	++
6	**2**	VnFe	Pentopan 500 BG	Chloroform	72	++
7	**2**	VnFe	Lipolase 100T	Toluene	72	–
8	**2**	VnFe	Lipolase 100T	Diisopropyl ether	72	–
9	**2**	VnFe	Lipolase 100T	Chloroform	72	++
10	**2**	TfeFe	Lipolase 100T	Chloroform	72	++
11	**2**	VnFe	Lipolase 100T	Methyl isobutyl ketone	64	+++
12	**2**	VnFe	Lipolase 100T	Acetonitrile	64	+++
13	**2**	VnFe	Lipolase 100T	*tert*-Butanol	72	++
14	**2**	VnFe	Ultraflo L	Acetonitrile	24[b]	–
15	**2**	VnFe	Lipolase 100L	Acetonitrile	24[b]	–
16	**2**	VnFe	Lipase A	Acetonitrile	80	–
17	**3**	VnFe	Pentopan 500 BG	Acetonitrile	64	+++
18	**3**	VnFe	Pentopan 500 BG	*tert*-Butanol	64	–
19	**3**	VnFe	Lipolase 100L	Acetonitrile	24[b]	–
20	**3**	VnFe	Lipolase 100T	Diisopropyl ether	24	–
21	**3**	VnFe	Lipolase 100T	Chloroform	63	+
22	**3**	VnFe	Lipolase 100T	Methyl isobutyl ketone	63	+++
23	**3**	VnFe	Lipolase 100T	Acetonitrile	63	+++
24	**3**	VnFe	Lipolase 100T	*tert*-Butanol	63	++
25	**4**	VnFe	Pentopan 500 BG	Toluene	72	+
26	**4**	VnFe	Pentopan 500 BG	Chloroform	72	++
27	**4**	TfeFe	Pentopan 500 BG	Methyl isobutyl ketone	72	++
28	**4**	VnFe	Pentopan 500 BG	Acetonitrile	88	++
29	**4**	VnFe	Pentopan 500 BG	*tert*-Butanol	72	+
30	**4**	VnFe	Lipase A	Acetonitrile	88	+
31	**4**	VnFe	Lipolase 100T	Chloroform	63	+++
32	**4**	VnFe	Lipolase 100T	Methyl isobutyl ketone	63	+++
33	**4**	VnFe	Lipolase 100T	Acetonitrile	64	+++
34	**4**	VnFe	Lipolase 100T	*tert*-Butanol	63	++

[a] Degree of acylation was estimated from intensity of spots after TLC: +, traces up to 25%; ++, 25–60%; +++, 60–95%.
[b] VnFe was completely hydrolysed due to presence of water in the enzyme preparation.



Scheme 1. Feruloylation of glycosides **1**–**4** by vinyl ferulate catalyzed by Lipolase 100T in organic solvents (Table 3).

24, 34). Generally, Lipolase 100T in more polar aprotic solvents like acetonitrile or methyl isobutyl ketone seems to be the best enzyme catalyst for feruloylation of glycosides with primary hydroxy group (entries 3, 12, 23, 33 or 11, 22, 32). It appears that rate of feruloylation of arabinofuranoside **1** under such conditions is about 1.5 times higher (entry 3) to pentopyranosides **2**–**4** (entries 12, 23, 33). The reactivity of pentopyranosides **2**–**4** is similar each to other. The reactivity of vinyl ferulate and 2,2,2-trifluoroethyl ferulate was comparable (entries 5 and 6 or 9 and 10).

The best results estimated from intensity of spots after TLC and summarised in Table 2 were repeated in preparative scale and products were isolated and characterised by NMR. The glycosides **5**–**8** feruloylated at primary position have been prepared in high yields under catalysis of Lipolase 100T in acetonitrile or methyl isobutyl ketone as solvents (Scheme 1, Table 3).

In a typical preparative run, glycoside (10 mmol), vinyl ferulate (0.33 g, 15 mmol, 1.5 equiv.) and Lipolase 100T (3 g) was added to 20 ml of acetonitrile or methyl isobutyl ketone at room temperature. The reaction mixtures were stirred at 37 °C for several hours (Table 3), then filtered, filter cake washed with acetone and combined organic phases were concentrated under reduced pressure. Crude mixtures were purified by silica-gel chromatography (ethyl acetate) to afford mono-*O*-feruloylated glycosides **5**, **6**, **7** and **8** (Table 3). Selected NMR data for primary protons: **5**, (300 MHz, CD_3OD): $\delta = 4.36$ (ddd, 2H, $J_{4,5} = 6.0$ Hz, $J_{4,5'} = 3.5$ Hz, $J_{5,5'} = 11.9$ Hz, H-5, 5′); **6**, (300 MHz, CD_3OD): $\delta = 4.39$ (ddd, 2H, $J_{5,6} = 5.8$ Hz, $J_{5,6'} = 2.2$ Hz, $J_{6,6'} = 11.9$ Hz, H-6, 6′); **7**, (300 MHz, CD_3OD): $\delta = 4.50$ (ddd, 2H, $J_{5,6} = 7.3$ Hz, $J_{5,6'} = 2.3$ Hz, $J_{6,6'} = 11.8$ Hz, H-6, 6′); **8**, (300 MHz, CD_3OD): $\delta = 4.37$ (ddd, 2H, $J_{5,6} = 7.5$ Hz, $J_{5,6'} = 5.1$ Hz, $J_{6,6'} = 11.4$ Hz, H-6, 6′).

Table 3
Preparative feruloylation of glycosides **1**–**4** under catalysis of Lipolase 100T in appropriate organic solvents

Glycoside	Solvent	Time (h)	Product	Yield (%)
1	Acetonitrile	62	**5**	83
2	Acetonitrile	64	**6**	64
3	Acetonitrile	69	**7**	53
4	Acetonitrile	67	**8**	63
1	Methyl isobutyl ketone	65	**5**	90
2	Methyl isobutyl ketone	65	**6**	49
3	Methyl isobutyl ketone	65	**7**	41
4	Methyl isobutyl ketone	65	**8**	88

In conclusion, cheap commercial enzyme preparations Lipolase 100T is capable to efficiently catalyse the feruloylation of various types of glycosides in non-aqueous organic environments. When the activated esters of ferulic acid serve as acyl donors, the acylation occurs at primary position in high yields within relatively short times. This transferuloylation activity of the studied enzyme preparations has never been reported before. Although previous reports [13–15] describe feruloylations of monosaccharides and glycosides, they suffer by lower yields as well as the high price [13] or lack of availability [14,15] of used biocatalysts. Thus, the enzymatic synthesis described here opens an attractive and economic way for preparation of mono-*O*-feruloylated pentofuranosides and hexopyranosides.

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