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Donor specificity and regioselectivity in Lipolase mediated acylations of methyl α-D-glucopyranoside by vinyl esters of phenolic acids and their analogues

Mária Mastihubová *, Vladimír Mastihuba

Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava, Slovakia

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ABSTRACT

Methyl α-D-glucopyranoside as a model acceptor was acylated by several phenolic and non-phenolic vinyl esters using immobilised Lipolase. Donor specificity and regioselectivity of reaction were investigated. Conversion and rate of acylation by structurally varied donors indicates that the synthetic reactivity of Lipolase corresponds to the hydrolytic activity of feruloyl esterase type A. Lipolase exhibited remarkable regioselectivity for primary position of methyl α-D-glucopyranoside. The acylation occurred exclusively at 6-*O* primary position when vinyl esters of phenolic acids (hydroxybenzoates, hydroxyphenylalkanoates and hydroxycinnamates) served as acyl donors (5–77%). In addition to the major 6-*O*-acyl products (52–79%), 2,6-di-*O*-acylated derivatives were isolated from reaction mixtures (2–13%) when non-phenolic donors were used (vinyl esters of fully methoxylated derivatives of phenolic acids, along with vinyl benzoates, cinnamates or some heterocyclic analogues).

Regioselective acylation of hydroxyls along the saccharide ring is constantly a fundamental challenge for organic chemists. The problem encountered in the chemical modification of sugar molecules may be avoided by introduction of biocatalytic procedures possessing high degree of regioselectivity in the acylation of polyhydroxylated substrates. Various hydrolases (lipases, esterases, proteases) have been introduced as catalysts for this purpose.[1–4]

Feruloyl esterases (FAEs) [E.C. 3.1.1.73] represent a group of carboxylic acid esterases catalysing hydrolysis of an ester bond between *p*-hydroxycinnamic acids and saccharides present in plant cell walls.[5–9] The classification of FAEs into four sub-classes (A–D) was proposed based on similarities in profiles of hydrolytic esterase activity against methyl esters of partly substituted hydroxycinnamic acids, their dimers, and protein sequence identities of the enzymes.[10] Recently, a new FAEs classification into 12 distinct families was proposed according to their sequences by descriptor-based computational analysis.[11] Based on the classification in terms of their substrate specificity, FAEs type A show a preference for the phenolic moiety of the substrate that contains methoxy substitutions. These enzymes appear to prefer hydrophobic substrates with bulky substituents on the benzene ring. Their protein sequences are more similar to sequences of lipases.[10] Regarding specificity against the hydrolysis of synthetic substrates, these FAEs hydrolyse methyl ferulate, methyl sinapate and methyl *p*-coumarate, but not methyl caffeate.

Thermomyces lanuginosus lipase (TLL)[12] possesses feruloyl esterase activity and displays a high level of sequence identity with FAE from *Aspergillus niger*[13,14] which was classified as FAE type A. TLL is the enzyme responsible for lipolytic activity of Lipolase®, a commercial lipase preparation supplied by Novozymes. Lipolase® is produced by a genetically modified strain of *Aspergillus oryzae*.[12,15]

Several reports describe enzymatic acylations of monosaccharides and oligosacharides,[16–21] as well as glycosides[20,22–28] (mainly flavonoids)[24–26] by phenolic acids and their aryl or arylalkyl analogues under action of lipases or FAEs to obtain substances with stronger antioxidant activities. Only few reports however studied regioselectivity[23] or enzyme specificity for wider scale of donors.[18,20,25] Donor specificity or regioselectivity of FaEs and relative lipases in acylations of saccharides by phenolic acids and their aromatic analogues is still not well understood. Some experimental conclusions of regioselectivity are questionable due to insufficient characterisation of products.

Most of reports describe use of the commercial lipase B from *Candida antarctica* (CALB) for successful synthesis of saccharide esters of phenolic acids.[20,22,24–27] Lipases from *Aspergillus niger*,[18] *Penicillium expansum*[23] or feruloyl esterase from *Sporotrichum thermophile*[16,17] were also applied with satisfactory results. Another commercial enzyme preparations with FAE activity were successfully applied in feruloylation of glycosides[28] or monosaccharides.[19] Generally, application of activated esters of phenolic acids in

* Corresponding author. Tel.: +421 2 59410246; fax: +421 2 59410 222.
E-mail address: chemjama@savba.sk (M. Mastihubová).

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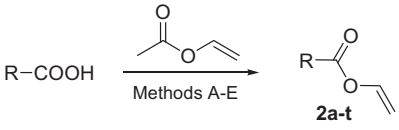
transesterifications appears to be more effective than the direct esterification of phenolic acids in respect of reaction time, donor/acceptor molar ratio and product yield. The available data for enzymatic acylation of saccharides with hydroxycinnamoyl donors were recently reviewed,[29] discussing the impact of organic solvent or ionic liquid systems on enzyme stability, selectivity and reaction yield.

Previously, a scale of commercial enzymes exhibiting feruloyl esterase activity has been tested in our laboratory for catalysis of transferuloylations on glycosides in organic solvents.[28] Lipolase 100T (immobilised TLL) was found to be the best preparation due to its ability to feruloylate different glycosides in satisfactory yields. This contribution presents results from our investigation of the reactivity of vinyl esters of several phenolic acids as well as their non-phenolic analogues as donors for enzymatic acylations of the model glycoside catalysed by Lipolase 100T. Regioselectivity of the reaction was also monitored.

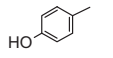
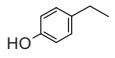
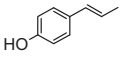
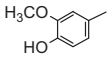
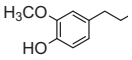
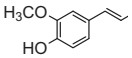
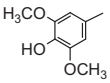
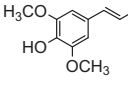
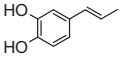
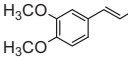
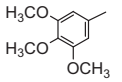
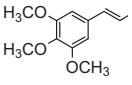
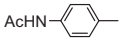
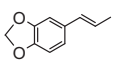
Methyl α-D-glucopyranoside (**1**) was selected as a good model acceptor which is frequently used in studies of regioselectivity of chemical[30–32] or enzymatic[33,34] acylations. The literature data indicate that reactivities of hydroxy groups of **1** during standing acylations catalysed by sterically more hindered bases like tertiary amines are in order 6-*OH* > 2-*OH* > 3-*OH* > 4-*OH*,[35] although the most reactive nucleophile of **1** is actually the hydroxyl 2-OH due to its activation by intramolecular hydrogen bond with the neighbouring *cis*-oriented anomeric α-OMe. Use of **1** could therefore help to reveal whether steric effects and electrostatic potential of the acceptor molecule could influence the relative reactivity of the enzyme. The potential disadvantage of using **1** as an acceptor is its lower solubility in CH_3CN, which may reduce yields of the acylated products.

Twenty vinyl esters **2a**–**t** were prepared from the corresponding acids to serve as donors for enzymatic acylations of Me α-D-Glc_p (Scheme 1). Phenolic vinyl esters **2a**–**k** were derived from 4-hydroxybenzoic, vanillic, syringic, gallic, 2-hydroxybenzoic, 4-hydroxyphenylacetic, 4-hydroxyphenyl-3-propionic, coumaric, ferulic, sinapic and caffeic acids. Their non-phenolic analogues **2l**–**t** were derived from benzoic, 3,4,5-trimethoxybenzoic, 4-acetylaminobenzoic, 2-thiophenecarboxylic, cinnamic, 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic, 3,4-(methylenedioxy)cinnamic and 3-(2-furyl)acrylic acids (Scheme 1).

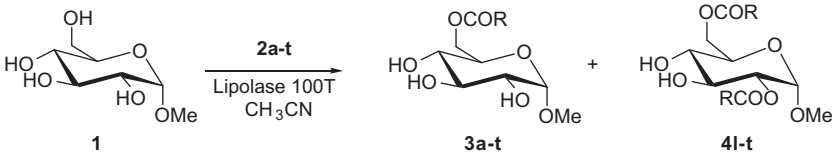
Most of the phenolic vinyl esters were synthesised by modified transesterification of the corresponding acids with vinyl acetate under catalysis of mercuric acetate and *p*-toluenesulfonic acid described previously by Gao et al. (Method A).[24] The competitive, undesirable acetylation of phenolic groups under these conditions was adequately controlled; the yields were however less satisfactory (39–68%). Preparation of vinyl esters catalysed by mercuric



Methods:
A. $Hg(OAc)_2$, p-TsOH, THF, rt, overnight;
B. $Hg(OAc)_2$, BF_3.OEt, THF, 40°C, 4h;
C. i) Ac_2O H_2SO_4, ii) Method B, iii) NH_2-NH_2.H_2O, CH_3CN;
D. i) Ac_2O, H_2SO_4, ii) Method B, iii) 3N HCl, THF;
E. $Pd(OAc)_2$, KOH, 40°C, 5 h.

Entry	R = aryl	Method/ Yield (%)	Entry	R = arylalkyl	Method/ Yield (%)	Entry	R = arylpropenyl	Method/ Yield (%)
a:		A/44	**f:**		A/53	**h:**		A/49
b:		A/42	**g:**		A/65	**i:**		A/68
c:		A/39				**j:**		A/42
d:		C/71				**k:**		C/73
e:		D/53				**p:**		B/73
l:		-				**q:**		B/77
m:		B/78				**r:**		B/75
n:		B/57				**s:**		B/88
o:		B/84				**t:**		E/82

Scheme 1.



Scheme 2.

Table 1
Acylation of methyl α-D-glucopyranoside by different phenolic vinyl esters (1.5 equiv) under catalysis of Lipolase 100T in acetonitrile at 37 °C

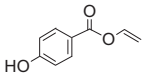
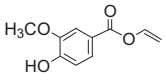
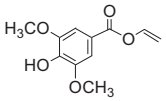
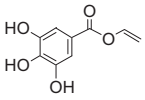
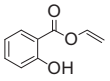
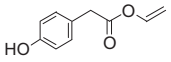
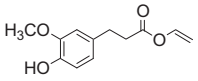
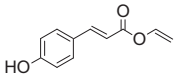
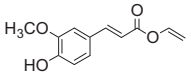
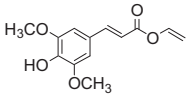
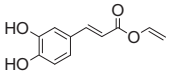
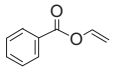
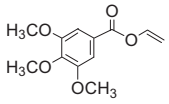
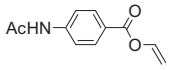
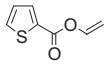
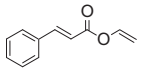
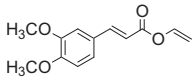
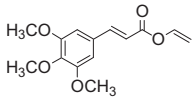
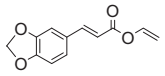
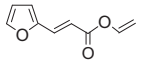
Entry	Donor (**2a**–**t**)	Time (h)	Isolated yield (%) 6-*O*-acyl (**3a**–**t**)
a		92	40
b		98	28
c		90	35
d		110	12
e		108	5
f		86	52
g		42	77
h		78	56
i		64	68
j		76	48
k		96	16

Table 2
Acylation of methyl α-D-glucopyranoside by different non-phenolic vinyl esters (1.5 equiv) under catalysis of Lipolase 100T in acetonitrile at 37 °C

Entry	Donor (**2a**–**t**)	Time (h)	Isolated yield (%)	
			6-*O*-Acyl (**3a**–**t**)	2,6-Di-*O*-acyl (**4l**–**t**)
l		98	76	13
m		72	63	5
n		67	65	2
o		72	52	7
p		96	79	7
q		72	73	6
r		70	71	7
s		67	73	5
t		67	55	5

acetate and BF_3 etherate (Method B) was modified according to the existing procedure.[36] This method was applied as an effective way only for synthesis of non-phenolic vinyl esters, since these conditions promote the uncontrollable acetylation of phenolic groups. The yields ranged from 57% to 88%. Direct vinylation of polyphenolic acids (gallic and caffeic) by the Method A or Method B failed. Therefore, the phenolic groups were protected by acetylation, protected acids were vinylated by the Method B and deacetylated by hydrazine hydrate (Method C). Overall yields (71% and 73%) of this three-stage synthesis were comparable to previous one-stage methods. The attempts to directly prepare vinyl salicylate either by Method A or Method B were unsuccessful, therefore the Method B was applied on acetylated salicylic acid and the product was deacetylated by 3 N hydrochloric acid in tetrahydrofuran (Method D). The acid-labile 3-(2-furyl)acrylic acid was vinylated under catalysis of palladium acetate and potassium hydroxide (Method E).[37] On the other side, direct vinylations of polyphenolic acids by Method E were also unsuccessful.

The prepared acyl donors **2a**–**t** (1.5 equiv) were used in studies of donor specificity, reactivity and regioselectivity of Lipolase 100T in transesterifications of glycoside **1** in acetonitrile (Scheme 2). Reactions were monitored by TLC until reaching the maximum concentration of products, stopped by filtration through Celite, the products were then purified by column chromatography and identified. Details of all synthetic steps and characterisation of products are provided in Supplementary data.

Survey of actual results of acylations of **1** with different phenolic donors summarised in Table 1 and with their non-phenolic analogues (Table 2) suggests a dependence of reaction rates, yields and regioselectivity on the donor structure. All activated acyls were readily soluble in acetonitrile. Some differences of reactivity of used donors in transesterification have to be therefore discussed. The reactivity decreased in the order: *p*-hydroxyphenylpropanoate > methoxylated derivatives and analogues of non-phenolic cinnamates ⩾ methoxylated derivatives and analogues of benzoates > derivatives of *p*-hydroxycinnamate > *p*-hydroxyphenylacetate > derivatives of *p*-hydroxybenzoate > caffeate ⩾ gallate > *o*-hydroxybenzoate.

These observations led us to conclude that character of the alkyl bridge between the aromatic ring and the carboxylic group of donors seems to play an important role in the transesterification reactivity of the studied lipase. When we compare decreasing reaction times and increasing chemical yields of analogical products **3a**, **3f**, **3h** as well as **3b**, **3i**, **3g** and **3c**, **3j**, we can see the influence of the length of the alkyl bridge on reactivity of phenolic donors in the reaction (Table 1). Prolonged central carbon chain enhance the reaction rates and yields of products, whereas absence of the chain decreases it. It is evident that changes in the distance between the ester bond and the aromatic ring influenced the synthetic performance of the enzyme. This phenomenon is not observed in reactions with non-phenolic donors, in which the yields do not fluctuate markedly (Table 2).

The chemical yields were strongly affected by the degree of hydroxylation or by position of hydroxyl on the aromatic ring. The 6-*O*-gallate **3d**, 6-*O*-caffeate **3k** and 6-*O*-salicylate **3e** were isolated in low yields. When empirically comparing the yields of **3d** < **3c** < **3m** or **3k** < **3i** < **3q** we can presume that the increasing degree of methoxy substitution of hydroxylated phenyl ring improves the yields of products.

Generally, one may assume that hydrophobic character of donors is very important for activity of Lipolase. This conclusion is also supported by our results with unsubstituted donors **2l**, **2p**, methylenedioxycinnamate **2s** and 4-acetylaminobenzate **2n**. Likewise, we used heterocyclic substrates **2o** and **2t** as uncommon donors and we observed that the enzyme accepts also heterocyclic ring in the donor structure.

Review of the presented results suggests that the donor specificity of Lipolase in transesterifications corresponds to the hydrolytic specificity of feruloyl esterase type A proposed by Crepin[10] or feruloyl esterase family 12 recently proposed by Udatha.[11] On the basis of these facts we can generalise that the substrate specificity in hydrolytic activity of type A FAEs correlates to their synthetic reactivity.

On the other side, the enzyme exhibited a strong regioselectivity toward α-D-glucopyranoside **1**. When vinyl esters of phenolic acids served as acyl donors, even when the most reactive *p*-hydroxyphenylpropanoate **2g** was used, the acylation occurred exclusively on 6-*O* primary position of methyl α-D-glucopyranoside (Table 1). On the contrary, some amounts (2–13%) of 2,6-di-*O*-acylated products **4l–t** were isolated from reaction mixtures in addition to the major 6-*O*-acyls **3l–t** when using non-phenolic donors (Table 2). It seems that the use of hydrophobic non-phenolic donors support a low level of diacylation. Despite lower regioselectivity of non-phenolic acylation, the yields of 6-*O*-acylated products **3l–t** are generally higher than the yields of the products **3a–k** 6-*O*-acylated with phenolic acids. The question is whether the additional acylation to position 2 is affected by the electrostatic potential of the acceptor or by more lipophilic character of molecule near the aglycon (methyl group).

Taking into account results of our previous work[28] we can state that Lipolase can accept a wide variation in structure of acyl donors and glycosyl acceptors; nevertheless it seems to have narrower specificity towards aromatic acyl donors than for sugar acceptors.

In conclusion, a series of phenolic and non-phenolic vinyl esters have been prepared and tested in enzymatic acylation of methyl α-D-glucopyranoside catalysed by Lipolase 100T. The influence of donor structure on enzyme activity, yield and regioselectivity of the reaction were evaluated. Lipolase can accept a wide variation in structure of acyl donors and their reactivity in transesterification correlates with the hydrolytic specificity of feruloyl esterase type A. Lipolase exhibited a remarkable regioselectivity in acylations of methyl α-D-glucopyranoside, especially with phenolic donors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.051.

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