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What can feruloyl esterases do for us?

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Abstract The role of feruloyl esterases in plant wall development, in gut health, and in the breakdown of plant biomass for the production of bioactive phytochemicals and biofuel is covered in this review. These enzymes have potential roles in stomatal cell function and the phenolic substitutions and cross-linkages between plant cell wall components. As more plant genomes are sequenced, the role of ferulic acid and feruloyl esterases *in planta* may be better understood. In human and ruminal digestion, these enzymes are important to de-esterify dietary fibre, releasing hydroxycinnamates and derivatives which have been shown to have positive health effects, such as antioxidant, anti-inflammatory and anti-microbial activities. They are also involved in colonic fermentation where their extracellular and intracellular activities in the microbiota improve the breakdown of polysaccharides and increase microbial production of short chain fatty acids. Their specificity can also be employed to synthesize bioactive compounds for cosmetic and health applications. The enzymatic disassembly of cereal straws is greatly enhanced when feruloyl esterase activity is present, although the substrate specificity of the esterase appears to have some bearing on its optimal application. The involvement of feruloyl esterases in the improved enzymatic and microbial saccharification of cereal-derived material demonstrates a high importance for these enzymes in animal feed preparation and bioalcohol production.

Keywords Phenolic acids · Human nutrition · Biofuel · Biomass breakdown · Enzymatic synthesis

Abbreviations

AnFaeA	Type-A feruloyl esterase from *Aspergillus niger*
AnFaeB	Type-B feruloyl esterase from *Aspergillus niger*
CA	Caffeic acid
ChlA	Chlorogenic acid
CLEA	Cross-linked enzyme aggregate
diFA	Diferulic acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
FA	Ferulic acid
FAE	Feruloyl esterase
GH10	Glycoside hydrolase family 10
GH11	Glycoside hydrolase family 11
HCA	Hydroxycinnamic acids
kDa	One thousand Dalton units
LDL	Low-density lipoprotein

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MCA	Methyl caffeate
MFA	Methyl ferulate
MpCA	Methyl *p*-coumarate
MSA	Methyl sinapate
pCA	*p*-Coumaric acid
SA	Sinapic acid
StFaeC	Type-C feruloyl esterase from *Sporotrichum thermosporum*
TsFaeC	Type-C feruloyl esterase from *Talaromyces stipitatus*
WB	Wheat bran
WS	Wheat straw

Introduction

Since the first review in 1998 (Williamson et al. 1998), there has been a plethora of review articles on feruloyl esterases, either as a subject on their own or as part of a review on xylanolytic enzymes (Faulds 2003; Matthew and Abraham 2004; Wong 2006; Fazary and Ju 2007, 2008; Topakas et al. 2007; Aurilia et al. 2008; Benoit et al. 2008; Koseki et al. 2009). These reviews describe the microbial source of feruloyl esterases, their purification and partial characterisation, specificity for synthetic and model substrates, etc. In particular, Fazary and Ju's 2008 review outlines patents where these esterases are involved. The structure, synthesis, location, nature of ester linkages, and interactions involving ferulates are covered elsewhere in this special issue and will not be dealt with in this article. In this review, I will concentrate on the role of feruloyl esterases in plant development, and human digestion, as well as a role for the enzymes in biomass processing.

Nomenclature and classification

Feruloyl esterases (E.C. 3.1.1.73), also known as ferulic acid esterases (FAE), cinnamoyl esterases, cinnamoyl ester hydrolases, and chlorogenate esterases, were originally classified into two groups based on their induction and substrate specificity (Kroon et al. 1996). Certain commercial preparations of cutinases and tannases contain ferulic acid (FA)-hydrolysing activity, with the *A. oryzae* tannase being able to hydrolyse 5,5′- and 8,5′-diFA diesters (Andersen et al. 2002; Garcia-Conesa et al. 2001). Although the crystal structure of feruloyl esterases from *Clostridium thermocellum* resemble cutinases (Prates et al. 2001; Schubot et al. 2001), it has yet to be shown if tannases and cutinases contain FAE-type side activities. Type-A FAE is inducible upon fungal growth on xylan and the enzyme preferred methoxy substitutions on the phenolic ring and a 1,5-linkage to arabinose, as found in graminaceous plants, while type-B was induced during growth on sugarbeet pulp and preferred hydroxyl substitutions. Subsequently, with the publication of gene/protein sequences, four putative types (A–D) were proposed based on sequence homology and biochemical analysis (Crepin et al. 2004). A further refinement to this classification has been proposed based on phylogenic analysis of known fungal genomes (Benoit et al. 2008) with seven subfamilies proposed, but containing only three biochemically characterised types of FAE (types A, B and C) while excluding those esterases classified previously as Type-D (Crepin et al. 2004).

The nomenclature used for feruloyl esterases in this review is as described previously (Faulds 2003), and based on that developed for glycoside hydrolases. It uses the letters of the esterase-producing micro-organism, followed by 'Fae' to designate it is an enzyme with feruloyl esterase-type activity, and then a letter to designate it proposed sub-type, based upon activity against the methyl esters of ferulic (FA), sinapic (SA), caffeic (CA) and *p*-coumaric acid (pCA). For example, the type-A esterase produced by *Aspergillus niger*, which has been called FAE-III (Faulds and Williamson 1994) and FAEA (de Vries et al. 1997) when first reported, should be now termed AnFaeA. Similarly, the putative type-B feruloyl esterase from *A. niger* (FAEB) (de Vries et al. 2002) would be renamed AnFaeB. Previously reported feruloyl esterases which have not been so thoroughly classified with regard to their substrate specificity cannot presently be defined by their sub-type.

Plant wall functionality

To date, little information is available about plant FAE. FA hydrolyzing activity has been detected in barley (Sancho et al. 1999; Humberstone and Briggs 2000;

Sun et al. 2005; Vanbeneden et al. 2007), but purification and characterization of the protein has not been fully reported, while a plant esterase exhibiting ferulic acid-hydrolysing activities has recently been purified from malted finger millet (Madhavi Latha et al. 2007). Most studies of FAE activity use model substrates, such as methyl ferulate (MFA). From using this substrate, hydrolytic activity decreased in barley grains during germination, suggesting a role for these enzymes in the developing grain, and the activity is heat labile (Sancho et al. 1999). In certain cases, it is incorrect to extrapolate activity on model substrates to what happens *in planta*, but barley extracts contain comparable enzyme activities to purified fungal enzymes in their ability to release of FA directly from cereal by-products (Sancho et al. 2001). In the Arabidopsis, rice and maize genome databases, only one entry for a carbohydrate esterase family 1 is listed for each genome (see CAZy database: http://www.cazy.org/). This is the same carbohydrate esterase family as all the identified fungal feruloyl esterases to date. These genes encode putative carboxylesterases, which in the case of the *Arabidopsis thaliana* protein has associated activity against model acetate substrates as do most of the characterized feruloyl esterases. It is thus possible that measured FA-hydrolyzing activity is in fact a side-activity of a general plant serine hydrolase. The isolation and sequence-specificity comparison of these plant proteins with fungal enzymes is thus required before we can ascertain if plants do produce feruloyl esterases. And if such specific enzymes are present, the location of such activities in the plant should also be determined.

Stomatal guard cells form and regulate the aperture of pores in the epidermis of most land plants, thus regulating the exchange of gases and water vapour between the external environment and the interior of the plant. Incubation of epidermal strips from leaves of *Zea mays* and *Vicia faba* (Jones et al. 2005) or *Commelina communis* (Jones et al. 2003) with an FAE or arabinanase impeded the function of guard cells surrounding the stomatal cell opening, while pectin degrading enzymes could reverse this action. The esterase could have a role in guard cell flexibility by modifying crosslinks between pectic arabinan and other cell wall polymers. Any feruloylation of the galactan side-chains of *C. communis* rhamnogalacturonan-I had no effect on stomatal function (Jones et al. 2005). It is unlikely that the esterases formed intermolecular cross-links, as suggested by these authors. As these studies involved microbial-derived enzymes, it is possible that a plant FAE will behave differently to fungal FAE on a plant wall structure, as is the case of pectin methylesterases on methyl-esterified pectins (Pelloux et al. 2007). Further research is also required to understand the role of putative plant FAEs in cell wall development and in their potential interactions with *in mura* peroxidases (Encina and Fry 2005) and feruloyl CoA transferases (Yoshida-Shimokawa et al. 2001) during the formation/cleavage of feruloyl cross-links.

Improved digestibility of cereals and grasses could also increase their potential as raw materials for biofuel production. Expression of the type-A FAE from *A. niger* (AnFaeA) in the forage grasses *Lolium multiforum* (Buanafina et al. 2006) and *Festuca arundinacea* (Buanafina et al. 2008) was explored in order to limit cell wall cross-linking and thus increase ruminal digestibility. The vast majority of AnFaeA-transformed plant lines showed a statistically significant decrease in FA and diferulate (diFA) in the walls relative to the control plants. If expression was specific to the vacuole, this decrease would not have occurred and so the results suggest that the esterase is not compartmentalised in a specific location. Three potential explanations were put forward to explain this: cell death during plant development, exocytosis, or incorrect targeting to the endoplasmic reticulum where it interfered with polysaccharide synthesis and feruloylation. Post-harvest digestion of the transformed plants with AnFaeA and a xylanase resulted in a considerable release of FA and diFA (5,5′, 8-*O*-4′ and 8,5′ benzofuran form), as well as a large amount of pCA. This suggests that AnFaeA was able to affect feruloylation patterns and cross-linking *in plana* making the modified cell wall prone to more extensive digestion through revealing more hydrolysis sites for the exogenously added enzymes. The ruminal digestion studies, however, were not so conclusive, showing that other factors are involved in dietary fibre breakdown (Buanafina et al. 2008).

The above studies suggest that endogenous feruloyl esterases may have an important role in plant development and are therefore targets for future plant engineering studies for industrial biomass. In the near future, further genetic and genome-specific information will be required in order to understand the role of

FAE in wall development, interactions with wall peroxidases, a potential role of intracellular FAEs in the mobilisation of the intracellular pool of phenolic conjugates in response to wounding/pathogen attack, and their regulation. This information will contribute to our understanding on how to control levels of important bioactive hydroxycinnamates and their derivatives for health and energy utilisation.

Dietary implications

Wheat bran (WB) has been one of the most effective sources of fibre in protecting against colon tumour development (Lupton 1999). Arabinoxylan fibre is not digested in the upper gut, but is rapidly fermented in the colon, producing predominantly acetate with a little butyrate in the proximal intestine in rat (Glei et al. 2006) and predominantly propionate in human (Hopkins et al. 2003). Studies have shown that a proportion of hydroxycinnamates (HCA) and diFA on the arabinoxylans can be cleaved by esterases within mucosa-free epithelial cells and released as free acids where they can then be absorbed into the circulatory system (Andreasen et al. 2001a, b). Figure 1 summarises information gathered from the research literature indicating the potential site of gut esterases releasing the specified phenolic acids. It is possible that intraepithelial serine hydrolases or more general carboxylesterases found in mast cells may be responsible for feruloyl esterase activity in the small intestinal mucosa (Huntley et al. 1985). This is similar to that described above concerning FA-releasing activity in plants. Larger feruloylated compounds, however, cannot be absorbed. Caco-2 cells have extra- and intracellular esterases able to de-esterify HCA and diFA esters (Kern et al. 2003). Methyl sinapate (MSA) was slowly metabolised compared to the methyl esters of FA, CA and pCA, which could be due to the specificity of the gut esterases as well as differences in uptake across the epithelial membrane. The 8-*O*-4′-diFA diester was completely hydrolyzed to the free form after 16 h while the 8,5′-benzofuran form is de-esterified at a much slower rate, with a larger proportion of the monoester remaining after 16 h than the fully free acid form. No free 5,5′-diFA was reported at this time. Esterified HCAs can enter intestinal cell models (Kern et al. 2003). A 3-fold increase in FA concentration in blood plasma was

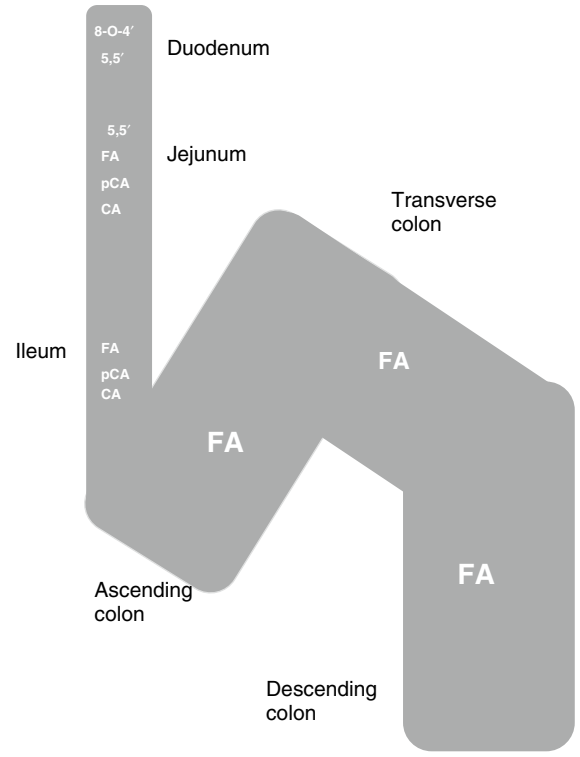

Fig. 1 Potential sites of feruloyl esterase activity in the human gastro-intestinal tract indicating reported phenolic acid release. Abbreviations: *CA* caffeic acid; *FA* ferulic acid; *pCA* *p*-coumaric acid; 5,5′, 5,5′-diferulic acid; 8-0-4′, 8-O-4′-form of diferulic acid

observed after whole grain breakfast cereal consumption (Constabile et al. 2008).

The in vivo physiological importance of FA depends on its availability for absorption. Approximately 95% of the total release of ferulate groups takes place during fermentation of WB in the human colon after release of feruloylated oligosaccharides by xylanases (Kroon et al. 1997). However, FA does not enter enterohepatic circulation, and oral or intravenous FA does not easily reach the colon. Due to the fact that insoluble and large soluble feruloylated polysaccharides/cell walls cannot transport through the epithelial wall, human cell-associated feruloyl esterases are possibly located on the brush border membrane (Andreasen et al. 2001a). FAE activity could also come from sloughed off epithelial cells. Figure 2 represents a cartoon of the putative transport of phenolic acids across the epithelial layer. Trace 5-*O*-caffeoylquinic (chlorogenic) acid hydrolysing activity was detected in the

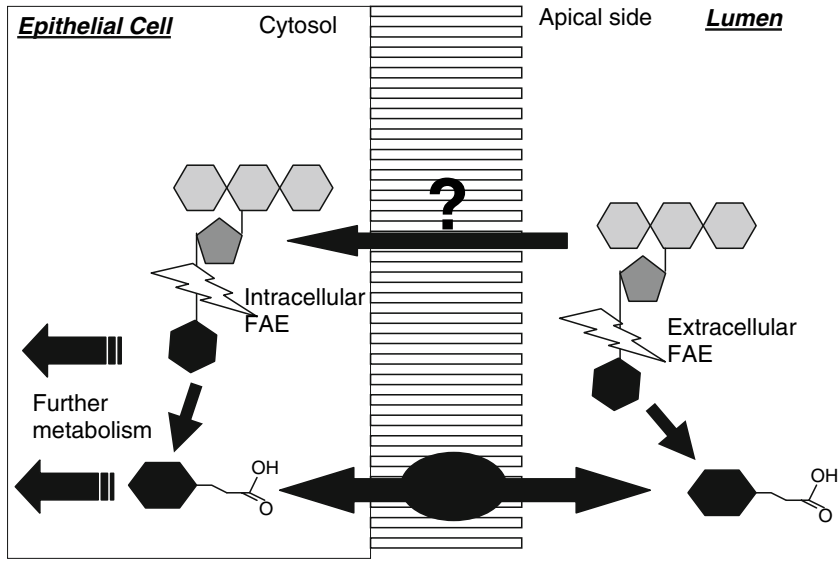

Fig. 2 Schematic gut epithelial cell showing feruloyl esterase activities

small intestinal mucosa of rats (Lafay et al. 2006). Chlorogenic acid (ChlA) is the main phenolic acid in the human diet. Human mucosal esterases can hydrolyse MCA but not ChlA (Andreasen et al. 2001a). It is also possible that FA in the colon is derived from the metabolism of CA after ingestion of ChlA-containing food. Chlorogenic acid is not hydrolysed by pancreatic secretions (Williamson et al. 2000) or extracts from human liver, small intestine or plasma (Plumb et al. 1999). Pancreatic cholesterol esterase and pancreatin were both found to hydrolyze sitostanyl ferulate and oryzanol (a mixture of hydroxycinnamate esters present in rice bran oil; Moreau and Hicks 2004). Pig liver esterase can hydrolyse HCA methyl esters (unpublished data) and porcine pancreatic esterases hydrolyse FA-sugar esters (Kato and Nevins 1985). Using the human hepatocarcinoma HepG2 cell model, no hydrolysis and thus no uptake of ChlA occurred (Mateos et al. 2006). For chlorogenic acid-derived CA to reach the liver, however, there thus must be a de-esterification process in the stomach or small intestine followed by further metabolism. While in vitro antioxidant activity of ferulic acid has been demonstrated, and xylo-oligosaccharides have in vitro prebiotic activities, there have been few or no apparent studies to perceive if the prebiotic/antioxidant activities of feruloylated xylo-oligosaccharides are altered upon hydrolysis, without the concomitant removal of FA from the reaction mix. This could help establish if conjugated FA-containing compounds are desired due to their improved solubility and processes designed to improve bioaccessibility and bioavailability. It must be noted, however, that very little evidence exists of an actual physiological benefit of FA in humans and ongoing research into this area has to also determine if FA is of benefit to our body through direct contact, through their role as radical scavengers in the insoluble fiber portion of our diet, or through the utilisation of these compounds by the gut microbiota and the metabolites produced. Once this is better understood, the role of endogenous FAE activity can be better evaluated.

The indigenous human gut microflora, including *E.coli, Lactobacilli* and *Bifidobacteria*, have been shown to produce FAEs. ChlA-hydrolysing activity was shown to be either cytoplasmic or internally membrane-associated (Couteau et al. 2001), which is similar to the proposed FAE activity in the human intestinal epithelial cells (Kern et al. 2003). Again, bacterial cell-associated esterase activity could represent a broad-specificity esterase rather than a specific FAE. There was a time-dependence production of different activities against MFA, methyl caffeate (MCA) and methyl *p*-coumarate (MpCA) (with no activity against MSA), with activity against chlorogenic acid appearing at an earlier stage of growth compared to MpCA/MFA activity (Couteau et al. 2001). This intracellular bacterial esterase activity is in contrast with the extracellular activity detected in human faecal slurries (Kroon et al. 1997; Vardakou et al. 2007). Only 3–7% of each of the

diferulates was released from WB by human faecal-derived extracts, showing that most of the diferulates which enter the colon still esterified to the polysaccharide matrix remain so (Vardakou et al. 2007). Pretreatment of WB with xylanase increased the amount of diFA released overnight by faecal bacteria to 40–80%. A 36 kDa FAE has recently been purified from the human gut-relevant species *Lactobacillus acidophilus* (Wang et al. 2004). This enzyme required xylanase-induced depolymerisation to occur in order to release FA, similarly with the aerobic fungal esterases.

The actual amount of FA released by the gut microorganisms is below the threshold required to exert a chemopreventative effect (Bathena et al. 2008). An alternative method has been tried involving the chemical coupling of FA to maize starch (Ou et al. 2001). The attached FA was released by the microbial FAEs in the colon at a rate almost twofold higher than with WB dietary fibre. Alternatively, augmentation of the enzymes produced by the gut microbiota with exogenous FAEs could improve the bioavailability. Encapsulated FAE-containing *Lactobacilli* have been prepared (Bathena et al. 2007, 2008) and studied using a human colonic model. A similar model was also used to show that arabinoxylans were broken down in the proximal colon by the action of inducible bacterial xylanases and feruloyl esterases (Vardakou et al. 2007). Non-feruloylated arabinoxylan did not induce FAE activity. FA-cross-linked arabinoxylans are more slowly fermented than non-cross-linked material (Hopkins et al. 2003). Feruloylation may impede arabinoxylan fermentation within the first few hours, as <10% FA is removed during this time, but not the overall utilisation by gut microbiota, with 85–95% free acid present after 24 h (Funk et al. 2007). This study also reported that 93–96% of diFA were removed from maize cell walls after 24 h incubation with human gut microflora, with no apparent specificity based on the structure of the dimer. Triferulates were also effectively removed.

Inducible extracellular FAE activity is widespread in ruminal microorganisms. Amongst ruminal xylanolytic *Clostridia* species, feruloyl esterase activity is mainly cytosolic (McSweeney et al. 1999) or essentially extracellular (Donaghy et al. 2000; Blum et al. 2000) demonstrating that the location of FAE can also vary according to the habitat of the producing species, even within a genus.

Feruloyl esterases in the synthesis of bioactive phenolic components

Hydroxycinnamates have widespread potential due to their antimicrobial, photoprotectant, antitumour and antioxidant properties (Graf 1992), as well as their use as flavour precursors (Walton et al. 2000). However, due to their relatively low solubility in aprotic media, applications for HCAs in oil-based food processing and cosmetics are limited. Their hydrophobicity could be enhanced by esterification of the carboxylic acid functional group with suitable groups, such as glycerol, alcohols or lipids. Lipases have been well studied as enzymatic tools to make esters, including HCA esters. A *Candida antarctica* lipase (both in the free form and immobilised in a packed bed reactor) transesterified mono-, di- and triacylglycerols from vegetable oils with ethyl ferulate for use as an ingredient for suncreams (Laszlo et al. 2003) or as antioxidant additives to cooking oils (Warner and Laszlo 2005). Lipases from *C. antartica, C. rugosa, Chromobacterium viscosum* and *Pseudomonas sp.* have been used to make vinyl ferulate conjugates with hydroxy-steroids and arbutin (Chigorimbo-Murefu et al. 2009). The products, especially the arbutin-ferulates, had increased antioxidant activities in the low-density lipoprotein (LDL) oxidation model. LDL is the major cholesterol carrier in the blood, and it is well established that an elevated plasma level of LDL is correlated with an increased risk of atherosclerosis and cardiovascular disease.

Esters of phenolic acids have been synthesised with pure and FAE-containing multienzyme mixtures. The feruloyl esterases from *Fusarium oxysporum* could catalyze the esterification of various phenolic acids to 1-propanol using surfactantless microemulsions (Topakas et al. 2003). AnFaeA transesterified MSA to 1- and 2-butyl sinapate (Vafiadi et al. 2008). SA and its derivatives have the potential to be used in anti-aging skin cosmetics, sun cream and agents to improve DNA repair (Moussou et al. 2004). They have also been implicated in cosmetic preparations for the treatment of skin diseases, stimulation of hair growth, as well as dietary ailments (Kosuga et al. 1998). Cross-linked enzyme aggregates (CLEAs) of *Humicola* enzyme preparations had a higher processing stability and synthetic activity than the free form without any detrimental effect on enzyme activity in the synthesis

of 1-butyl ferulate (Vafiadi et al. 2008). Similarly, AnFaeA in either free or CLEA form was used to catalyze the acylation of one of the primary hydroxyl groups of glycerol with SA using ionic liquid mixtures (Vafiadi et al. 2009). The synthesised sinapoylated glycerol was a slower antioxidant when compared to free SA in the LDL oxidation model system. AnFaeA has also been shown to esterify FA and SA to glycerol as well as to monomeric sugars (Tsuchiyama et al. 2006). Feruloyl glycerol was shown to be an effective scavenger of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, but again lower than that measured for free ferulic acid (Tsuchiyama et al. 2006). It has not yet been established why these HCA glycerols are poorer antioxidants than their corresponding free acids. HCA glycerol derivatives are more water-soluble than FA and thus have a wider range of applications in the food, beverage and cosmetic industries.

Feruloylated carbohydrates and their fragments also have potential uses, and fungal enzyme preparations have been used for the transacylation of various glycosides at their primary hydroxyl group in a non-aqueous environment leading to high yields in relatively short times (Mastihubová et al. 2006). The type-C TsFaeC could perform a transesterification reaction to yield feruloyl arabinose from MFA and L-arabinose (Vafiadi et al. 2006a) and StFaeC showed a strong preference for short chain alkyl ferulates, such as MFA (Vafiadi et al. 2006b). Lipases could not catalyse these reactions due to an electronic and/or steric effect (Otto et al. 2000). Oligosaccharides containing arabinofuranosyl residues with blocked reducing ends, such as feruloylated arabinose, are potential inhibitors of an α-(1,5)-arabinosyltransferase involved in the assembly of mycobacterial cell wall arabinan (Pathak et al. 2002; Vafiadi et al. 2007).

It is interesting to note that most of the feruloyl esterases examined to date as tools for the synthesis of putative bioactive compounds show a similar specificity for the substitutions around the phenolic ring for synthetic reactions as they display for hydrolytic reactions.

Deconstruction of plant cell walls and potential uses in biofuel supply

In the breakdown of plant tissue, the use of feruloyl esterases can aid glycoside hydrolases in the release of free carbohydrates for subsequent fermentation (also as occurs during digestion), as well as releasing phenolic acids as valuable by-products. In general, FAEs alone do not produce either high levels of free FA nor do they significantly influence changes in biomass structure/solubility. A table giving yields of FA recovered from plant biomass by the action of FAEs and xylanases is given in Shin et al. (2006), and so such information will not be repeated here.

Plant tissue type is a major influence on how biomass is degraded by enzymes. Leaf sheath and stem pith tissues from corn stover released more FA upon treatment with a cellulase and a FAE than other maize tissues (Anderson and Akin 2008). For the stems themselves, the thinner-walled pith cells are easily degraded. In cereal brans, FAEs substantially degrade the aleurone layer of maize fibre with little structural change occurring in the pericarp (Anderson and Akin 2008). Thus it is important that enzyme preparations used for the substantial breakdown of plant biomass contain feruloyl esterase activity. But there is still research to be carried out to determine if there is a preference for the type of FAE utilised?

Activity of FAEs is limited predominantly to the location of the feruloyl groups on the polymer, the conformation of feruloylated arabinoxylan, and/or interactions between the feruloylated polysaccharides and other wall components. It was recently shown that family GH11 xylanases were the generally preferred synergistic partners with FAEs for the release of FA from insoluble biomass, while family GH10 xylanases were preferred for the liberation of the 5,5′-dimer (Faulds et al. 2003, 2006). There was no obvious general preference for the FAE acting with a particular family of xylanases in releasing FA from water-unextractable arabinoxylan. Feruloyl esters were not a problem in the hydrolysis of WB aleurone and nucellar layers by a GH11 xylanase, but the pericarp stayed intact (Beaugrand et al. 2004), suggesting diferuloylation and arabinose branching is a more important impediment to a better utilisation of cereal brans. Enzymatic release of FA from brewer's spent grain (BSG) appears to augment the action of other glycoside hydrolases in addition to xylanases in comparison to the two enzyme system on WB, suggesting a more complicated interaction between wall polymers in barley compared to wheat (Faulds et al. 2002).

Additional catalytic and non-catalytic proteins aid feruloyl esterases in performing de-esterification

reactions. AnFaeA alone released a small amount of FA from WB in 24 h (6%), but when fused at the N-terminus to swollenin, a non-catalytic fungal protein which aids in the breakdown of cellulose fibres, this amount doubled (Levasseur et al. 2006). The supernatant of the transformed *T. viride* culture expressing the fusion protein released 45% of the alkali-extractable FA within 4 h, showing the need for other activities, such as xylanase, for the release of FA. When AnFaeA is fused at the C-terminus to a xylanase, 100% of the total FA was released from WB in 4 h (Levasseur et al. 2005). It is thought that a better synergistic effect on cereal brans is due to the physical proximity of the hydrolytic esterase and the xylanase together with specific substrate targeting through inclusion of an additional binding domain. These studies also demonstrate that modifications at the N- and C-terminus of AnFaeA do not affect its catalytic ability.

In the production of fuel ethanol, a pretreatment step is currently recommended to free the carbohydrates from the aromatic components of the cell wall. Warm season grasses, such as maize, sugarcane, bermudagrass and switchgrass, are especially high in phenolic acid esters (Akin and Chesson 1989). Slow to partial degradation patterns in Coastal bermudagrass coincides with the presence of phenolic acids (Anderson and Akin 2008). It is most likely that the esterase-freed phenolic acids will require removal from the hydrolysate prior to further saccharification and fermentation. Such a removal may be economically viable due to the potential functionality of FA. The presence of lignin interacting with the polysaccharides increases the complexity of the wall and the proportion of lignin in a plant tissue is related to its digestibility. It has been long postulated that FAEs are involved in cleavage of lignin-carbohydrate linkages. Figure 3 shows the structures of monolignols in comparison with the hydroxycinnamates. An ester of pCA and coniferyl alcohol has been synthesised as a representative of the ester linkage between hydroxycinnamic acid and lignin monomers (Benoit et al. 2006), where AnFaeB, a type-C FAE, had a greater affinity and catalytic efficiency for this substrate than did the type-A diFA-cleaving AnFaeA. Hydrolysis of wheat straw (WS) is hampered by the complex interaction between cellulose (~40% of the dry mass), hemicellulose (~25%) and lignin (~20%). Alone, AnFaeA and AnFaeB caused significant release of FA and pCA from steam-exploded WS, with the type-C esterase, AnFaeB, being the more effective esterase (Benoit et al. 2006). AnFaeA was more effective when combined with a cellulase and xylanase (Tabka et al. 2006), with enhanced hydrolysis at 50°C. This elevated temperature suits the glycoside hydrolases rather than the esterase. Addition of a non-ionic surfactant, such as Tween 20, enhanced hydrolysis further, with over 80% of the initial glucose in the WS recovered in the hydrolysate. Similarly, a combination of the same three enzymes were found to significantly release of nearly three quarters of the sugars from oat hulls in 24 h (Yu et al. 2003).

Significant increases in the depolymerisation of hot water-pretreated corn stover cellulose were observed with the cellobiohydrolase Cel7A, a xylanase, a feruloyl esterase (PfFaeA) and an acetylxylan esterase (Selig et al. 2008). Esterase and Cel7A alone resulted in a 37% increase in glucan conversion to

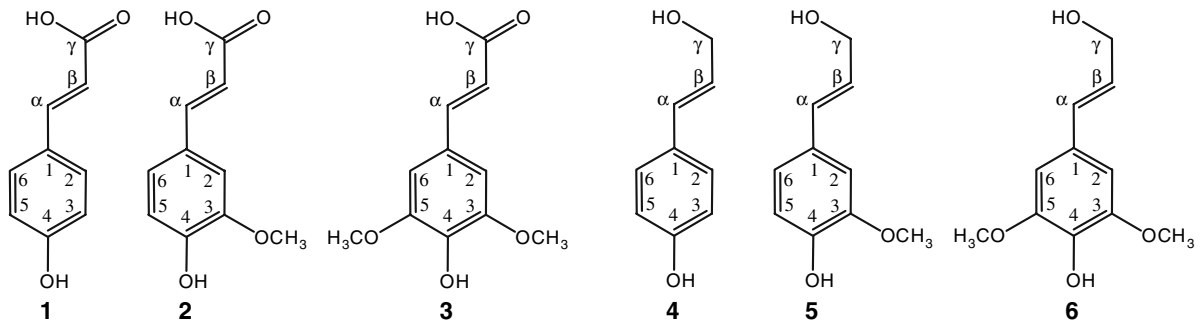

Fig. 3 Hydroxycinnamates and monolignols. *1* *p*-coumaric acid; *2* ferulic acid; *3* sinapic acid; *4* *p*-coumaryl alcohol; *5* coniferyl alcohol; *6* sinapyl alcohol

cellobiose compared to Cel7A alone, and a 52% increase in xylan converted to xylobiose. It is also possible that FA linking hemicellulose to lignin is also cleaved by the action of the esterases with or without the xylanase.

Ensilage is the anaerobic process of preserving moist crops by lactic acid fermentation, which in turn often improves animal performance. A number of ensilaging lactic acid bacteria produce feruloyl esterases and these strains substantially increased in situ ruminal fibre degradation, an effect consistent with silo fermentation of the animal feed (Nsereko et al. 2008), but the pattern and extent of improved digestibility depends on the specific forage fed to the animal (Krueger et al. 2008).

Use of feruloyl esterases has also been reported in other industrial processes, such as the utilisation of straws in paper manufacturing (Tapin et al. 2006; Record et al. 2003) and detoxifying jojoba meal to make it suitable for animal feed (Laszlo et al. 2006). Coffee and cider industry by-products contain high quantities of ChlA: 2.7 g/kg in coffee pulp and 330 mg/kg in apple marc (Benoit et al. 2006). Only AnFaeB was able to release CA and pCA: 100 and 73%, respectively, from coffee pulp, and 83 and 34%, respectively, from apple marc, while AnFaeA had no significant effect.

Conclusions

Feruloyl esterases are lesser understood members of the phenolic-acting enzyme family, which include peroxidases, laccases, polyphenoloxidases, etc., but they have a potentially valuable impact across many areas of plant processing, from improving the bioavailability of phytonutrients from the foods we eat to processing aids for sustainable energy supply. Compared to main-chain-acting glycosyl hydrolases, oxido-reductases, lipases and proteases, there is not a great deal known about feruloyl esterases, but their potential for opening up the plant cell wall is significant for designing processes for improved biomass utilisation. With limited specificity and structural studies performed on extracellular microbial enzymes, the opportunity to rapidly improve our knowledge on these enzymes should be taken. The rapidly expanding interest in lignocellulosic biomass utilisation together with the awareness of natural, health-promoting components in foods, such as the hydroxycinnamates and derivatives, warrants a better understanding of the physiological role of these (microbial, human epithelial and commensal bacterial) enzymes in breaking down plant structures during digestion and how we can manipulate their activities for improved biotechnological and nutritional impact. We need further research to know the range of substrates these enzymes can work on as well as structural studies to understand how they work and how we can improve them as biocatalysts. An understanding of how these enzymes can be induced in the colon and regulated will help design functional foods and colon delivery methods involving feruloylated polysaccharides, or if necessary contribute to the development of processing aids to facilitate ferulate release. And then there is the role of feruloyl esterase-type activity in the plant cell wall itself and potential roles in plant defence and phenolic turnover. It took 10 years to have the second Ferulate meeting, but the interest in these plant components, and the enzymes associated with their biosynthesis and breakdown, suggests that such conferences will need to be held more frequently.

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