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Feruloyl Esterase

A Key Enzyme in Biomass Degradation

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Abstract

Feruloyl esterase forms a part of the enzyme complex that acts collectively and synergistically to completely hydrolyze xylan to its monomers. The enzyme has found potential uses in a wide variety of applications of interest to the agrifood and pharmaceutical industries. This review describes the enzymology of feruloyl esterases involved in xylan degradation. The occurrence of feruloyl esterases in various microorganisms and their physiochemical properties are presented. The nature of the enzyme substrates and products, the role of synergistic interactions with xylanases and other accessory enzymes, as well as the sequence-structure relating to the reaction mechanism are emphasized.

Index Entries: Feruloyl esterase; ferulic acid and crosslinks; feruloylated oligosaccharide; hydroxycinnamic acid; glucurono arabinoxylan.

Introduction

The biorefining of crop components–starch, grain fiber, and crop residues–to fermentable substrates for the production of high-value products such as ethanol, butanol, lactic acid, and citrate represents a renewable energy source. Much of the research in this area has been motivated by environmental, economic, and national security concerns to develop an alternative source of fuels now mostly derived from petroleum. The current fuel ethanol capacity reaches approx 4.5×10^9 gal, with most of the production based on fermentation of glucose derived from the breakdown of starch. Agricultural residues (straws, hulls, stems, stalks), deciduous and coniferous woods, and herbaceous energy crops containing lignin, hemicellulose, and cellulose constitute an untapped resource of fermentable sugars for fuel production. The degradation of lignocellulosic materials represents a challenge because the individual components have different

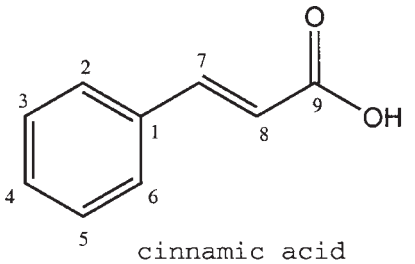
degrees of resistance to hydrolysis, and their relative composition varies, depending on the source materials *(1,2)*. Biologic pretreatment with microorganisms to solubilize lignocellulosic materials has been extensively studied *(3)*. The potential for fungal pretreatment to increase digestibility of the substrate is owing to the ability of the fungal enzymes to disrupt plant cell walls, resulting in partial breakdown of the lignin/carbohydrate complex. Enzyme degradation of any one of these biomass components needs the combined and synergistic action of several enzymes. Complete hydrolysis of the hemicellulose fraction requires two groups of enzymes: (1) endoxylanase and β-xylosidase, which cleave the xylan main chain; and (2) accessory enzymes, which remove the side chains and break crosslinks between xylan and other plant polymers. The latter group consists of α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterases, and feruloyl esterase *(4)*. Among the accessory enzymes, feruloyl esterases play a key role in enhancing the accessibility of enzymes to and subsequent hydrolysis of hemicellulose fibers by removing the ferulic acid side chains and crosslinks.

Feruloly esterases (FAEs) (EC 3.1.1.73) (ferulic acid esterases, cinnamoyl esterases, cinnamic acid hydrolases, *p*-coumaroyl esterases, hydroxycinnamoyl esterases, and so on) belong to a subclass of carboxylic esterases (EC 3.1.1). The enzyme cleaves ester bonds between hydroxycinnamic acids esterified to arabinoxylans (AXs) and certain pectins present in plant cell walls. This enzyme reaction is one of the controlling factors for increasing the extent of degradation of lignocellulosic biomass for bioenergy conversion, and the manufacture of pulp and paper. A wide range of bacteria and fungi has been reported to secrete the enzyme, which is highly inducible, depending on the growth substrates *(5)*.

Occurrence of Ferulates, Diferulates, and Triferulates in Cell Walls

Ferulic acids are covalently linked to polysaccharides, including glucuronoarabinoxylans (GAXs), xyloglucans, and pectins, through ester linkages. Ferulic acid is a cinnamic acid with the chemical name (3-methoxy-4-hydroxy)-3-phenyl-2-propenoic acid, or 3-methoxy-4-hydroxy-cinnamic acid (Fig. 1). Another cinnamic acid, *p*-coumaric acid, is also present in smaller amounts in plant cell walls. Both ferulic and *p*-coumaric acids are mainly present in the *trans* isomeric form *(6)*. The methyl esters of these cinnamic acids are frequently used as synthetic substrates for FAE activity assay.

In the commelinoid group of monocotyledons, including the orders Arecales (palms), Commelinales (spiderwort, kangaroo paw), Cyperales (sedges such as papyrus, cotton grass, sawgrass), Poales (barley, corn, millet, rice, wheat), and Zingiberales (ginger, arrowroot), ferulic acids are associated with GAXs in the primary cell wall *(7)*. The commelinoid group contains approximately half of all monocotyledon families, and most research has been done on the Poales. In the cell walls of vegetative tissues of grasses and cereals, the xylan chain is substituted with arabinose and



Substituents			
C3	C4	C5	
H	OH	H	**p-coumaric acid**
OH	OH	H	caffeic acid
OCH_3	OH	H	**ferulic acid**
OCH_3	OH	OCH_3	sinapic acid

Fig. 1. Chemical structures of hydroxycinnamic acids.

glucuronic acid and, hence, is called GAX. However, in the aleurone and starchy endosperm of cereal grains, the xylan chains are substituted almost exclusively by arabinose with very little glucuronic acid and are thus referred to as AX, a term often used in the literature relevant to ferulic acid chemistry (Harris, P. J., personal communication, Feb. 18, 2005).

GAX consists of β(1→4)-linked xylose chains that carry glucuronyl and arabinosyl substituents attached at the C(O)2 and C(O)3, respectively, to the xylose residues *(8)*. In cereals, such as wheat, corn, and barley, ferulic acid is mainly linked via its carboxylic acid group to the C(O)5 position of the arabinofuranose in the xylan chain (Fig. 2). In bamboo, ferulic acid is also crosslinked to xyloglucans via the C(O)4 position of xylose side chain residues *(9)*.

In barley grain, the outer layers (husks, pericarp, testa, and aleurone cells) contain 0.6–0.9% dry wt of total phenolic acids. The highest concentration of ferulic acid and *p*-coumaric acid is found in fractions containing high levels of aleurone cells and husks, respectively *(10,11)*. In wheat bran, ferulic acid and *p*-coumaric acid account for 0.66 and 0.004% dry wt of the cell wall, respectively *(12)*. Isolated aleurone cells from wheat contain 1.84% (w/w) of the cell walls and comprise ferulic acid (90%), *p*-coumaric acid (10%), and minor amounts (0.006%) of diferulic acid *(13)*. Corn bran cell wall contains ~3% (w/w) ferulic acids, compared with ~1% in sugar-beet pulp *(14)*.

In dicotyledons, ferulic acid has so far been identified only in the primary cell walls in the family Chenopodiaceae (of the order Caryophyllales), such as spinach and sugar beet *(15,16)*. In these plants, ferulic acids are ester linked to pectic polysaccharides, mainly at the C(O)6 of galactosyl and the C(O)2 or C(O)3 position of arabinosyl side-chain residues (Fig. 3). The dicots generally contain smaller amounts of hydroxycinnamic acids compared to monocots.

A considerable proportion of the ferulates form dimers (dehydrodimers and cyclodimers) through either the aromatic ring or the aliphatic side group, resulting in polysaccharide-polysaccharide and polysaccharide-lignin crosslinks (Fig. 2). Dimerization is achieved by peroxidase-catalyzed oxidative coupling *(17)*. Phenolic acid bridges between polysaccharides and lignin have a general structure of the ferulic acid aromatic ring ether

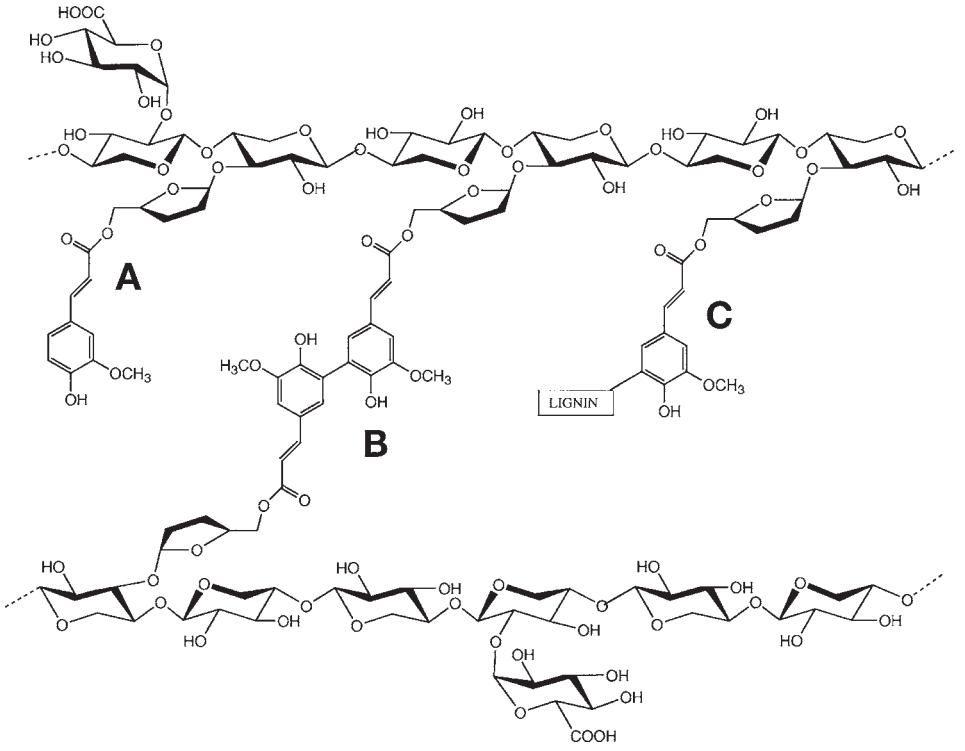


Fig. 2. Feruloylation of GAX, which is main carbohydrate component of many hemicelluloses from primary cell walls of commelinoid group: **(A)** feruloyl group attached to C(O)5 of arabinofuranosyl residue; **(B)** diferuloyl crosslinked C(O)5 of arabinofuranosyl residues (5-5′ dimer); **(C)** feruloyl group attached to C(O)5 of arabinofuranosyl residue and lignin.

linked to lignin and the carboxylic group ester linked to polysaccharide *(10)*. In wheat straw, ether-linked ferulic acid accounts for 1.1% dry wt of alkali-extracted lignin and might explain the high solubility of Poaceae (formerly Gramineae) lignins in NaOH *(19)*. It has been shown that ferulates couple with both coniferyl and sinapyl alcohol monomers in ryegrass lignins *(20)*. Ferulic acids also mediate polysaccharide-protein crosslinks via tyrosine or cysteine residues *(21)*. It has been demonstrated that kinetically controlled reactions between ferulic acid and the tripeptide Gly-Tyr-Gly with horseradish peroxidase (HRP) and H_2O_2 result in dehydropolymerization and a range of heterooligomers *(22)*. HRP-mediated crosslinking between feruloylated AXs and β-casein has also been reported *(23)*.

Recent investigations suggest that radical coupling of ferulates does not stop at the dimer stage but results in higher ferulic acid oligomers. A number of ferulic acid trimers have been isolated from corn bran *(24,25)*. The finding of these trimers implies that the reactions and formations of crosslinking in the cell wall can be more extensive than previously recognized.

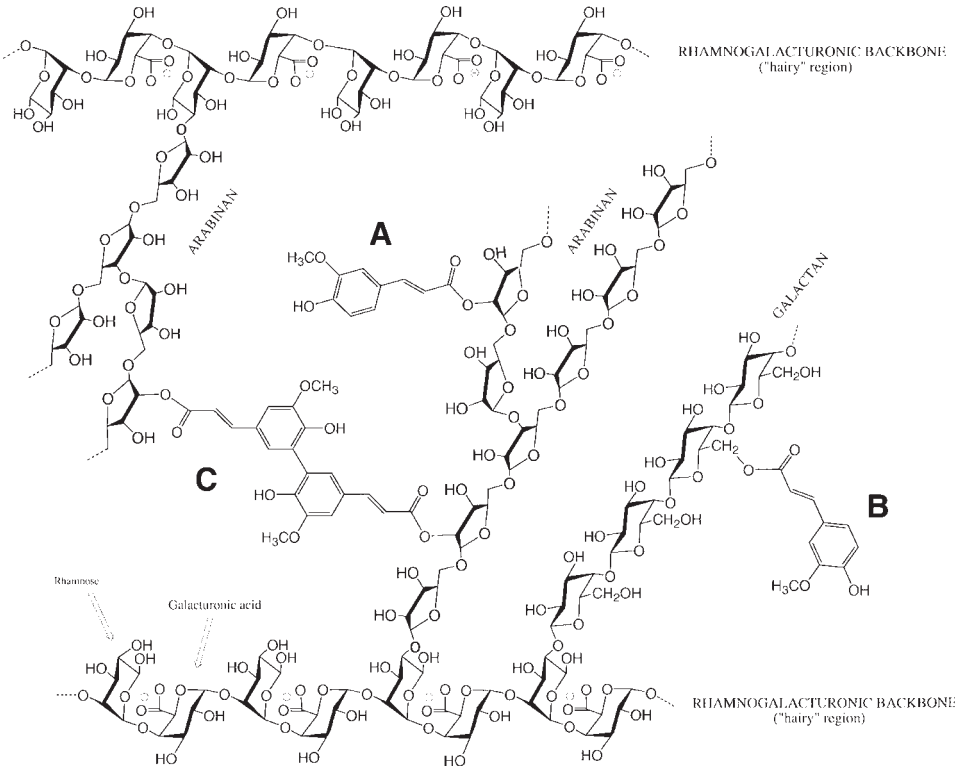


Fig. 3. Feruloylation of rhamnified pectin from sugar beet, showing rhamnogalacturonic backbone ("hairy" region): **(A)** feruloyl group attached to C(O)2 of arabinofuranosyl residue; **(B)** feruloyl group attached to C(O)6 of galactopyranosyl residues; **(C)** diferuloyl group attached to C(O)2 of arabinofuranosyl residues (5-5′ dimer). The "smooth" region of pectin, comprising α-1,4-linked galacturonic acid residues, is not shown.

Structures of Ferulic Acid Crosslinks

The polysaccharides to which ferulic acid is crosslinked and the position of attachment can be determined by the treatment of feruloylated polysaccharides in plant cell walls with a mixture of exo- and endoglycanases, followed by isolation and structural determination of the feruloyl oligosaccharides obtained from the enzyme digest. Knowledge of the molecular structures of feruloyl oligosaccharides is needed to reveal how ferulic acids are attached to the main chain polymers, and also how they form bridges in linking various polysaccharide chains together. Such information is a critical part in understanding the action of feruloyl esterases on the disassembly of natural polymeric substrates. Ferulic acid substitution of cell wall polysaccharides of wheat bran has been identified as 2-*O*-[5-*O*-(*trans*-feruloyl)-β-L-arabinofuranosyl]-D-xylopyranose (FAX), with the ferulic ester linkage at the 2-*O*-arabinosyl branch points of the xylan backbone *(12)* (Fig. 4). The most abundant feruloyl compound, *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-

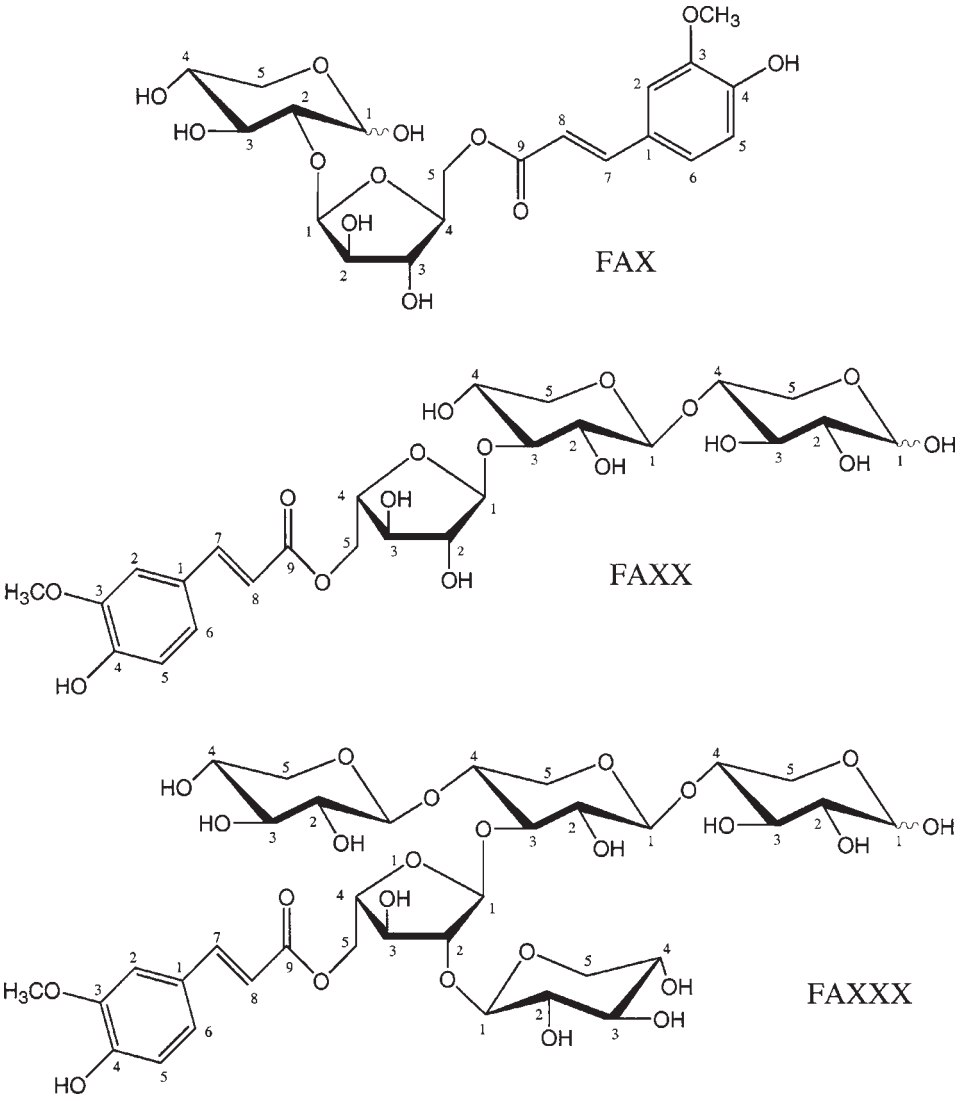


Fig. 4. Feruloylated oligosaccharides isolated from plant cell walls. FAX: 2-*O*-[5-*O*-*trans*-feruloyl)-β-L-arabinofuranosyl]-D-xylopyranose; FAXX: *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1->3)-*O*-β-D-xylopyranosyl-(1->4)-D-xylopyranose; FAXXX-I: [5-*O*-(*trans*-feruloyl)-*O*-β-D-xylopyranosyl-(1→2)-*O*-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose; FAXXX-II: *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose. PAX, PAXX, PAXXX: *p*-coumaric acid is substituted for ferulic acid in the above structure (not shown).

D-xylopyranose (FAXX), has been identified in the cell wall of corn shoot *(26)*, pineapple cell walls *(27)*, and barley straw *(28)*. The latter also contains *p*-coumaric acid ester linked to arabinose, as *O*-[5-*O*-(*trans*-*p*-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (PAXX). In growing bamboo shoots, ferulic acid is covalently associated

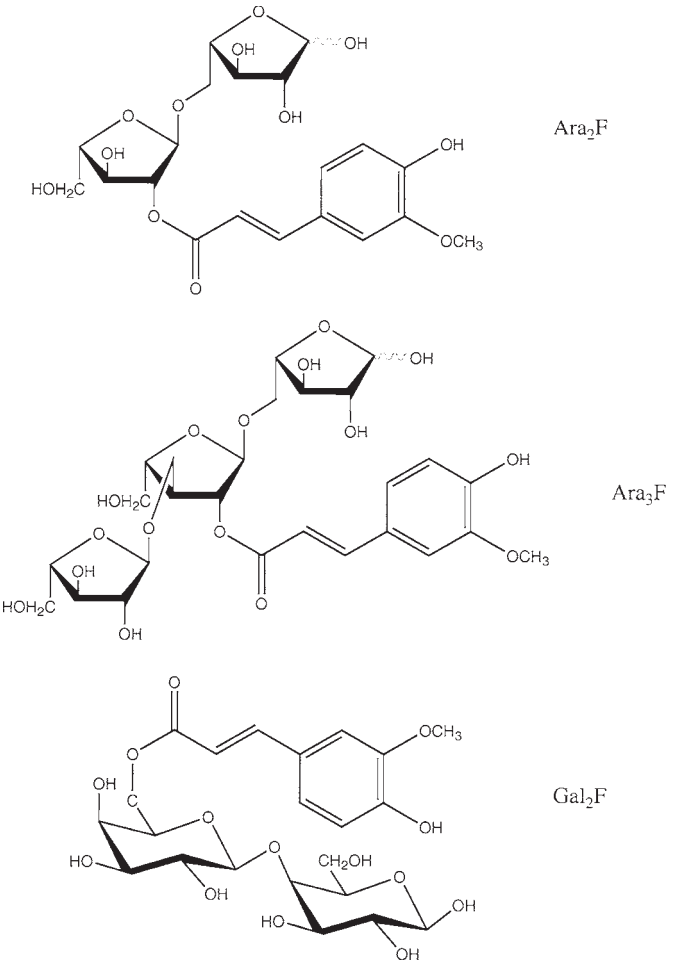


Fig. 5. Feruloylated oligosaccharides isolated from sugar-beet pulp. Ara_2F: feruloylated arabinose disaccharide, *O*-[2-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→5)-L-arabinofuranose; Ara_3F: arabinose trisaccharide, *O*-α-L-arabinofuranosyl-(1→3)-*O*-[2-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→5)-L-arabinofuranose; Gal_2F: feruloylated galactose disaccharide has the structure of *O*-[6-*O*-(*trans*-feruloyl)-β-D-galactopyranosyl]-(1→4)-D-galactopyranose

with xyloglucan in the cell wall hemicellulose, with the structure of *O*-[4-*O*-(*trans*-feruloyl)-α-D-xylopyranosyl]-(1→6)-D-glucopyranose *(9)*. Also isolated from bamboo shoot are the *p*-coumaroylated AX trisaccharides (PAXX); as well as a tetrasaccharide, *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXXX) *(29)*. FAXXX has also been isolated from sugarcane bagasse *(30)*.

In sugar-beet pulp, feruloylated oligosaccharides are derived from pectic neutral side chains containing arabinose or galactose residues *(16,31)* (Fig. 5). Feruloyl groups are linked to C(O)2 of the arabinose in both the

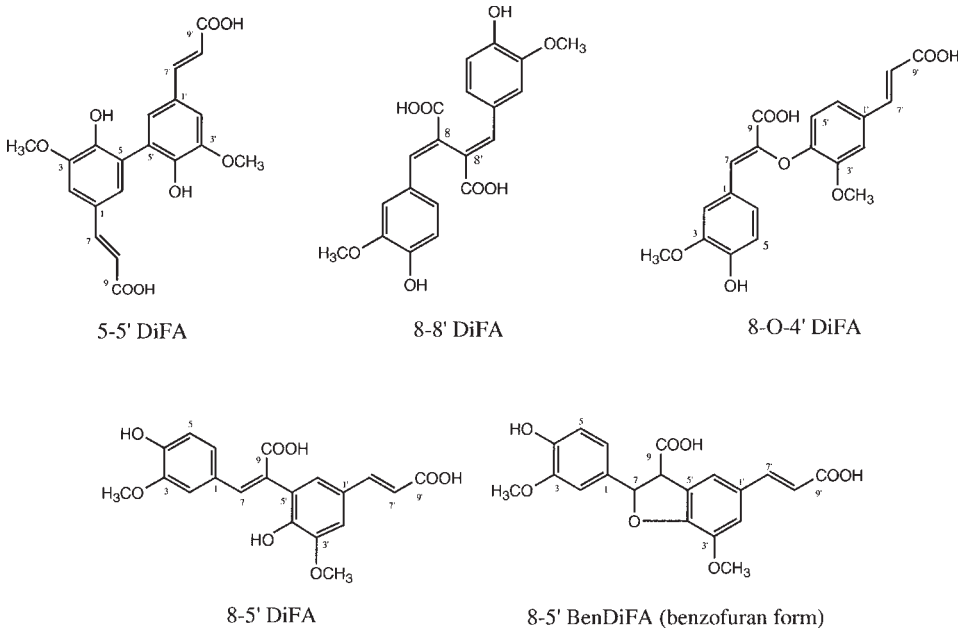


Fig. 6. Ferulic acid dimers: 5-5′-DiFA, (*E,E*)-4,4′-dihydroxy-5,5′dimethoxy-3′-bicinnamic acid; 8-5′-DiFA, (*E,E*)-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamic acid; 8-5′-BenDiFA (benzofuran form), *trans*-5-[(*E*)-2-carboxyvinyl)]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid; 8-8′-DiFA, (4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid); 8-*O*-4′-DiFA, (*Z*)-β-{4-[(*E*)-2-carboxyvinyl)]-2-methoxyphenoxy}-4-hydroxy-3-methoxy-cinnamic acid.

feruloylated arabinose disaccharide *O*-[2-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→5)-L-arabinofuranose, and the arabinose trisaccharide *O*-α-L-arabinofuranosyl-(1→3)-*O*-[2-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→5)-L-arabinofuranose. In addition, feruloylated galactose disaccharide has the structure of *O*-[6-*O*-(*trans*-feruloyl)-β-D-galactopyranosyl]-(1→4)-D-galactopyranose. In the walls of rapidly growing cell suspension cultures of spinach cells, the major esters contain a feruloyl arabinobiose, *O*-[3-*O*-(*trans*-feruloyl)-α-L-arabinopyranosyl]-L-arabinose and a feruloyl galactobiose, 4-*O*-[6-*O*-(*trans*-feruloyl)-β-D-galactopyranosyl]-D-galactose *(32)*.

Four dehydrodimers have been isolated and purified from rye bran after alkaline hydrolysis (Fig. 6). The major product is (*Z*)-β-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxyl}-4-hydroxy-3-methyoxy-cinnamic acid (abbreviated 8-*O*-4'-DiFA). Also present are 5-5′ DiFA, (*E,E*)-4,4′-dihydroxy-5,5′dimethyoxy-3′-bicinnamic acid; 8-5′ DiFA, (*E,E*)-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamic acid; 8-5′ DiFA benzofuran form (8-5′-BenDiFA), and *trans*-5-[(*E*)-2-carboxyvinyl)]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid, in the order of increasing amounts *(33)*. In saponified extracts of wheat bran and sugar-beet pulp, 5-5′ DiFA, 8-O-4′ DiFA, and 8-5′ BenDiFA have been detected *(34)*. Another

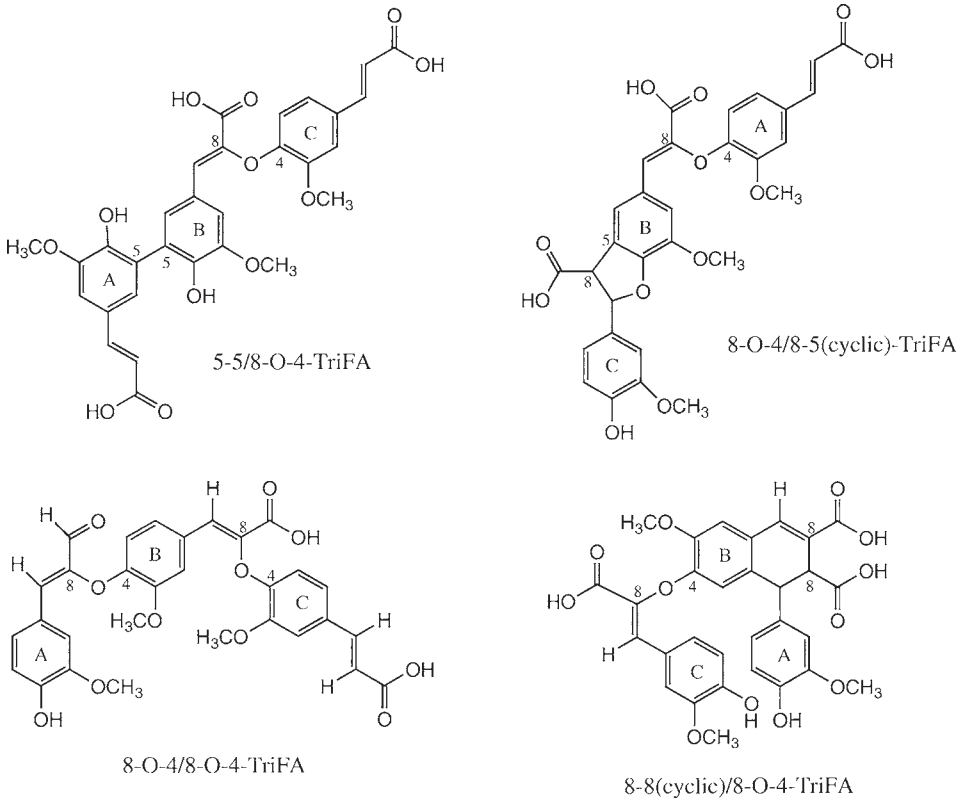


Fig. 7. Ferulic acid trimers: 5-5/8-*O*-4-dehydrotriferulic acid, 8-*O*-4/8-5(cyclic-dehydrotriferulic acid, 8-*O*-4/8-*O*-4-dehydrotriferulic acid, 8-8(cyclic)/8-*O*-4-dehydrotriferulic acid.

dimer, 8-8′-DiFA (4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid), has been identified in the enzymatic hydrolysate of sugar-beet pulp after saponification *(14)*. The amount of dehydrodiferulic acids accounts for 0.14 and 2.5% (w/w) of the enzyme digest of sugar-beet pulp and corn bran, respectively, suggesting a high degree of crosslinking of the heteroxylans in the bran cell wall of corn. It has been calculated that each heteroxylan macromolecule contains approx 75 esterified ferulic acid groups and approx 30 diferulic bridges. A spectrum of dehydrodiferulic acids, including 8-5′, 8-8′, 5-5′, 8-*O*-4′, and 4-*O*-5′ DiFAs, has been identified from whole grains of corn, wheat, spelt, rice, wild rice, barley, rye, oat, and millet. The majority is in the insoluble dietary fiber fraction *(35)*. The level of total DiFA ranges from 0.24 to 1.26% of the insoluble fiber, with the 8-5′ DiFA the dominant species. It has been postulated that ferulic radical couplings to monolignols (coniferyl or sinapyl alcohol monomers) function as nucleation sites for the lignification process *(17)*. There are indications that diferulate crosslink reduces the rate and, to a lesser degree, the extent of enzyme hydrolysis of cell walls *(36)*. In systems using cell walls from corn cell suspension containing ferulates and H_2O_2, the 8-5′ derivative was identified as the major

form generated from oxidative coupling, accounting for 45% of the dehydrodimers *(37)*. Using fungal laccase and HRP for oxidative crosslinking between feruloylated AXs, the major dimers formed were 8-5′ benzofuran, 8-8′ and 8-*O*-4′ *(38)*.

Dehydrotriferulic acids isolated from corn bran have been characterized to contain varying combinations of 5-5, 8-5, 8-8, and 8-*O*-4 linkages (Fig. 7). These include 4-*O*-8/5-5-dehydrotriferulic acid, 8-*O*-4/8-5(cyclic)-dehydrotriferulic acid, 8-*O*-4/8-*O*-4-dehydrotriferulic acid, and 8-8 (cyclic)/8-*O*-4-dehydrotriferulic acid *(25,39,40)*. The detection of trimers with 8-5, 8-8, and 8-*O*-4 linkages is the first indication that ferulic acids may function in coupling three carbohydrate chains. However, trimers containing 5-5 linkage need not implicate the crosslinking of three carbohydrate chains, because 5-5 coupling may form intramolecularly *(41)*.

Classification of Feruloyl Esterases

Feruloyl esterases have been classified as either type A or type B, based on their specificity for aromatic substrates and their varying ability to release diferulic acids from esterified substrates *(42)*. Recently, a more elaborate classification based on substrate utilization and supported by primary sequence identity has been proposed to consist of four subclasses: type A, B, C, and D *(43)*. Type A is active on methyl ferulate (MFA), methyl *p*-coumarate (M*p*CA), and methyl sinapate (MSA). These FAEs have sequences related to those of lipases and are able to hydrolyze synthetic ferulate dehydrodimers. Examples of this group of enzymes include *Aspergillus niger* FAE-A (AnFaeA). Type B FAEs are specific against MFA, M*p*CA, methyl caffeate (MCA), but not MSA. These enzymes do not release diferulic acid and show sequence similarities to carboxylic esterase family 1 - acetyl xylan esterase. *Penicillium funiculosum* FAE-B and *Neurospora crassa* FAE-I belong to this group. Type C and D act on all four hydroxycinnamic acid methyl esters. Type C enzymes do not release diferulic acids from model and complex substrates, whereas type D enzymes are able to hydrolyze dimers. Type C and D show sequence similarities to chlorogenate esterase and xylanase, respectively. Type C includes *A. niger* FAE-B (AnFaeB) and *Talaromyces stipitatus* FAE-C. Type D enzymes include *Piromyces equi* EstA and *Celluvibrio japonicus* Esterase D. There are too few sequences and three-dimensional structures available to substantiate fully the proposed classification at this stage. Future refinement is needed with acquisition of more research data on the sequence/structure and biochemical properties of feruloyl esterases.

Feruloyl Esterases From Microbial Sources

All microbial feruloyl esterases are secreted into the culture medium. The presence of an enzyme that hydrolyzes ester linkages of ferulic acid from the crude hemicellulose preparation of wheat bran was first reported as a component of the cellulolytic and xylanolytic systems of *Schizophyllum*

commune (44). The enzyme feruloyl esterase (FAE) was later isolated and purified to homogeneity from *Streptomyces olivochromogenes* with an apparent molecular mass of 29 kDa; pH and temperature optima of 5.5 and 30°C, respectively; K_m of 1.86 m*M*; and V_{max} of 0.3 µmol/(min·mg) using methyl ferulate as substrate *(45)*. FAE activity was also detected in the culture filtrate of *S. avermitilis* CECT3339 *(46)*. The enzyme exhibited a pH optimum at 6.0 with stability between 6.0 and 8.0, and an optimum temperature of 50°C with stability between 30 and 40°C.

Aspergillus *Fungi*

Early studies have predominately focused on the two major feruloyl esterases isolated from *A. niger*, namely, AnFaeA (previously FAE-II and FAE-III) and AnFaeB (formerly CinnAE and FAE-I) *(47–49)*. AnFaeA has been cloned into and expressed by *Pichia pastoris,* with yields up to 300 mg/L *(50)*. FAE-A is monomeric (29 kDa), with a p*I* of 3.6, whereas AnFaeB is a dimer (132 kDa) with a p*I* of 3.0. FAEs isolated from *A. oryzae* and *A. awamori* show physicochemical properties very similar to those of AnFaeA *(51,52)* (Table 1).

AnFaeA is specific for ferulic and sinapic acid methyl esters, and AnFaeB is specific for ferulic, *p*-coumaric, and caffeic acid methyl esters. Both enzymes prefer the arabinose residue to which ferulic acid is attached in the furanose form when acting on feruloylated oligosaccharides from sugar-beet pulp and wheat bran. The enzymes exhibit maximum activity on feruloylated trisaccharides. An increase in chain length of the substrate (degree of polymerization [dp] > 3) reduces the rate of hydrolysis *(15)*. AnFaeA acts only on the ferulic acid attached to C(O)5 of the arabinofuranosyl residues (as in xylan) and C(O)6 of galactose (as in pectin), not on ferulic acids linked to C(O)2 of arabinose (also in pectin), whereas AnFaeB has no clear preference for the two types of linkages. Both enzymes degrade sugar-beet pectin and wheat AX by releasing ferulic acid but have opposite preferences; AnFaeA is more active than AnFaeB toward wheat AX, whereas AnFaeB is more active toward sugar-beet pectin *(5)*.

Clostridium *Bacteria*

In *Clostridium thermocellum*, two bifunctional enzymes, Xyn10A and Xyn10B, containing domains corresponding to xylanase and feruloyl esterase activities have been identified in the cellulosome, a multiprotein complex with high efficiency in the hydrolysis of lignocellulosic substrates *(53,54)*. XynY and XynZ have been recently reclassified as Xyn10B and Xyn10A, respectively *(55)*.

The *xyn10A* gene contains 2511 bp encoding 837 amino acids with a molecular mass of 92 kDa. The sequence organization consists of FAE at the N-terminus, which is bridged to a xylanase domain via a proline-rich linker, a carbohydrate-binding domain, and a dockerin domain. The nucleotide sequence of *xyn10B* contains an open reading frame of 3228 bp encoding a protein of 120 kDa. Xyn10B displays a modular organization similar to that

Table 1
Physiochemical Properties of Feruloyl Eserases From Microbial Sources[a]

Microorganism/reference	Enzyme	Molecular mass (kDa)	p*I*	Optimum pH (stability)	Optimum temperature (stability)	K_m (m*M*)	V_{max} (μmol/ [min·mg])	K_{cat} (s^{-1})	Substrate
Aspergillus niger (47,48)	AnFaeA	29	3.6	5.0	55–60	1.11 ± 0.2	0.67 ± 0.07		MFA (pH 5.3, 50°C)
						0.47 ± 0.09	0.20 ± 0.01		M*p*CA
						1.20 ± 0.24	3.21 ± 0.28		MSA
	AnFaeB	132 (dimer)	3.0			1.21 ± 0.08	2.03 ± 0.06		MFA (pH 5.2, 50°C)
						0.15 ± 0.06	5.88 ± 0.30		M*p*CA
						0.43 ± 0.11	5.99 ± 0.34		MCA
Aspergillus oryzae (51)	FAE	30	3.6	3.0–7.0 (4.5–6.0)	45				
Aspergillus awamori (52)	FAE	35	3.8	5.0 (4.0–11.0)	45 (up to 50)				
Clostridium thermocellum (56)	FAE_ Xyn10A	90	5.8	4.0–7.0 (3.0–10.0)	(up to 70)	5	12.5		FAXXX (pH 6.0, 60°C)
	FAE_ Xyn10B	116							
Sporotrichum thermophile (110)	StFAE-A	33	3.1	6.0 (5.0–7.0)	55–60	0.71 ± 0.07		3.30	MFA (pH 6.0, 45°C)
						0.09 ± 0.002		5.47	M*p*CA
						0.21 ± 0.02		4.56	MCA
Streptomyces olivochromogenes (45)	FAE	29	7.9, 8.5	5.5	30	1.86	0.3		MFA (pH 5.5, 30°C)
Neocallimastix strain MC-2 *(60)*	FAE-I	69	4.2	6.2 (5.5–6.8)	(40)	0.032	2.9		FAXX (pH 6.2, 40°C)
	FAE-II	24	5.7	7.0 (6.4–7.6)	(40)	0.010	11.4		FAXX (pH 7.0, 40°C)
Pseudomonas flourescens supsp. *cellulosa (73,74)*	XylD	58.5				2.1 ± 0.1	5.7 ± 0.3		MFA (pH 6.0, 37°C)
						0.84 ± 0.1	2.6 ± 0.3		$Ara_1F(1,5)$
						0.56 ± 0.08	3.9 ± 0.25		Xyl_2Ara_1F
						0.26 ± 0.04	2.7 ± 0.6		Xyl_3Ara_1F
Neurospora crassa (42)	FAE-B	35		6.0 (6.0–7.5)	55 (up to 55)	0.25	8.97	12.19	MFA (pH 6.0, 37°C)
						0.021	20.87	12.19	M*p*CA
						0.02	8.20	4.80	MCA
						0.46	10.34	6.04	Ara_2F

Fusarium oxysporum (111,112)	FAE-I	31	9.5	7.0 (7.0–9.0)	55 (up to 30)	0.60 ± 0.03		6.85	MFA (pH 6.0, 45°C)
						0.20 ± 0.01		19.88	M*p*CA
						1.12 ± 0.05		0.47	MSA
						0.26 ± 0.02		5.92	MCA
	FAE-II	27	9.9	7.0 (7.0–9.0)	45 (up to 45)	0.58		39.4	MFA (pH 6.0, 45°C)
						0.68		13.0	M*p*CA
						0.29		116.6	MSA
						0.81		7.9	MCA
Penicillium expansum (76)	FAE	57.5		5.6	37	2.6	27.1		MFA (pH 6.0, 37°C)
						2.9	18.6		M*p*CA
Penicillium funiculosum (75)	FAE-B	35.5				0.047		16.0	MFA (pH 6.0, 37°C)
						0.007		24.2	M*p*CA
						0.139		24.1	MCA
Piromyces equi (64)	EstA	55.5		6.7 (5.8–7.7)	50–60 (30–70)	0.004	56.3		FAXX (pH 6.0, 37°C)
						0.075	14.7		Ara-F

[a] Ara_1F = 5-*O*-(*trans*-feruloyl)-L-arabinofuranose; Xyl_2Ara_1F = *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose; Xyl_3Ara_1F = *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose; Ara_2F = *O*-[2-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→5)-L-arabinose. MFA = methyl ferulate; M*p*CA = methyl coumarate; MSA = methyl sinapate; MCA = methyl caffeate.

of Xyn10A, but in reversed order with the FAE domain (286 amino acid residues) at the C-terminal end. The recombinant feruloyl esterase domain of Xyn10A obtained by overexpression in *Escherichia coli* has been characterized *(56)*. The enzyme has an optimum pH in the range of 4.0–7.0 and temperature stability up to 70°C. Using FAXXX as the substrate, K_m and V_{max} values are 5 m*M* and 12.5 μmol/(min·mg), respectively (pH 6.0, 60°C).

An extracellular feruloyl esterase has been isolated and purified from the thermophilic *C. stercorarium* grown on birchwood xylan *(57)*. The purified esterase, with an apparent mass of 3.3 kDa, releases ferulic, *p*-coumaric, caffeic, and sinapinic acid from the respective methyl esters, and also ferulic acid from destarched wheat bran. The enzyme has optimum activity at pH 8.0 and 65°C, with K_m and V_{max} values of 40 μ*M* and 131 μmol/(min·mg) for the hydrolysis of methyl ferulate.

Ruminal Microbes

Anaerobes in the rumen are known to efficiently degrade lignified cell walls, and many have FAE activity *(58)*. High levels of esterases acting on *p*-coumaroyl and feruloyl AX have been found in culture filtrates of *Piromyces* MC-1; *Neocallimastix* MC-2; and *Orpinomyces* PC-1, PC-2, and PC-3 *(59)*.

Two extracellular FAE-Is and FAE-IIs from the anaerobic fungus *Neocallimastix* strain MC-2 have been purified and characterized *(60)*. FAE-I (68 kDa, p*I* 4.2, optimum pH of 6.2) hydrolyzes both FAXX and PAXX, but FAE-II (24 kDa, p*I* 5.7, optimum pH of 7.0) is specific for FAXX. The apparent K_m and V_{max} values for the hydrolysis of FAXX have been measured to be 31.9 μ*M* and 2.9 μmol/(min·mg), respectively, for FAE-I, and 9.6 μ*M* and 11.4 μmol/(min·mg), respectively, for FAE-II, at optimum pH and 40°C. A third enzyme, which is a dimer of 11 kDa, has substrate specificity for *p*-coumaroyl oligosaccharides. It shows 100 times more activity toward PAXX than FAXX, with K_m and V_{max} values of 19.4 μ*M* and 5.1 μmol/(min·mg) at pH 7.2 and 40°C, respectively *(61)*.

The genes encoding CinA and CinB from the ruminal bacterium *Butyrivibrio fibrisolvens* E14 have been expressed at high levels in *E. coli (62)*. CinA releases ferulic acid from para[5-*O*-(*trans*-feruloyl)-arabinofuranose] prepared from wheat bran. CinA and CinB show 28% amino acid identity, with predicted molecular mass of 27 and 31 kDa, respectively.

The multidomain enzyme XynE from *Ruminococcus flavefaciens* contains, from the N-terminal end, a family 11 endoxylanase, a stabilizing domain, a dockerin-like region, a T-rich linker, and a phenolic acid esterase domain similar to that identified from *Clostridium thermocellum* XynA *(63)*. (For glycosylase classifications, refer to http://afmb.cnrs-mrs.fr/CAZY/.)

A module cinnamoyl ester hydrolase (EstA) isolated from the anaerobic fungus *P. equi* comprises a docking domain, a linker, and a catalytic

domain *(64)*. EstA is active against synthetic and plant cell wall substrates and acts synergistically with xylanase. The enzyme forms a part of a multiprotein cellulose-binding cellulase-hemicellulase complex.

Induction of Expression of Feruloyl Esterases

Expression of the FAE genes in *A. niger* is under the control of a complex system of transcriptional regulation. The *faeA* gene together with *xlnB*, *xlnC*, and *xlnD*, the three xylanase genes encoding the main xylanolytic enzymes, as well as other genes for accessory enzymes, is controlled by the transcriptional activator XlnR *(65)*. This *xlnR* gene, which encodes a zinc binuclear cluster protein, is a member of the GAL4 family of transcription factors *(66)*. The end product of xylan degradation, xylose, at concentrations >1 m*M* represses the xylanolytic genes, including *faeA*, mediated by the carbon catabolite repressor protein CreA *(67)*. In addition to this xylan/xylose system, a ferulic acid–specific system of induction is involved. Aromatic compounds with a defined structure, such as ferulic acid and vanillic acid, induce the production of AnFeaA in *A. niger* grown on spelt xylan or wheat bran *(68,69)*. All the inducing compounds tested contain a benzene ring with C-3 methoxy and C-4 hydroxy groups but no substitutions at C-5 *(70)*. In the ruminal bacterium *B. fibrisolvens* E14, the expression of CinB is repressed by CinR, which binds to the *cinR-cinB* intergenic region and also to the neighboring regions of the promoters *(71)*.

Release of Ferulic Dimers by Feruloyl Esterases

Two feruloyl esterases have been identified to release diferulic acid in the degradation of plant cell walls and, therefore, potentially break down xylan-lignin crosslinks. *Pseudomonas fluorescens* XylD or *A. niger* AnFaeA, alone or in concert with a xylanase, release the ferulate dimer 5-5′-DiFA from barley and wheat cell walls *(72)*. At low concentrations, AnFaeA and XylD release 93 and 36% of the saponifiable 5-5′-DiFA, respectively, from solubilized wheat bran, but only 12 and 15%, respectively, from solubilized sugar-beet pulp. AnFaeA at higher concentrations releases 8-*O*-4′-DiFA from solubilized wheat bran, but not sugar-beet pulp *(34)*. The XylD enzyme also liberates ferulic acid from feruloylated oligosaccharides of destarched wheat bran and releases acetic acid from acetylxylan. The enzyme contains a cellulose-binding domain (CBD) at the N-terminus, in addition to the catalytic esterase domain *(73,74)*. Another enzyme, *P. funiculosum* FAE-B, consists of a carbohydrate-binding domain that binds crystalline cellulose *(75)*. The enzyme is efficient in hydrolyzing hydroxycinnamic acids from synthetic and plant cell wall–derived ester substrates, such as ferulate and *p*-coumarate. A feruloyl esterase purified from *P. expansum* releases ferulic acid and *p*-coumaric acid from methyl esters of the acids FAXX and PAXX *(76)*.

Synergistic Actions With Xylanase and Other Accessory Enzymes

The synergistic action between FAE and xylanase and other accessory enzymes in the degradation of plant cell wall polysaccharides has been well established. Feruloyl esterases from *A. niger*, similar to many other xylanolytic accessory enzymes, act synergistically with xylanases and other enzymes in the group. AnFaeA releases ferulic acid from destarched wheat bran only in association with xylanase. AnFaeB releases a small amount of free ferulic in the absence of xylanase, but the amount of hydrolysis increases with the addition of xylanase *(47)*. AnFaeA releases ferulic acid from wheat bran at a level 24-fold higher when a *Trichoderma viride* xylanase is added in the incubation *(77)*. Synergism between these two enzymes results in a significant increase in the amount of reducing sugars in the digestion of oat hulls, an agricultural byproduct containing a high concentration of ferulic acids *(78,79)*.

The source of xylanase as well as the type of FAEs used in combination influences the amount of ferulic acid released; family 11 xylanases are more efficient in the hydrolysis of ferulic acid, and family 10 xylanases exhibit a more synergistic effect on the release of diferulic acid *(80)*. In the synergistic degradation of insoluble wheat flour AX, FAE does not affect the mode of action of xylanases. The increased release of ferulic acid is the result of the xylanase action producing short-chain feruloylated xylooligosaccharides, which are more accessible as FAE substrates *(81)*. In the degradation of wheat AX, the release of ferulic acid by AnFaeA depends largely on the degradation of the xylan backbone by endoxylanase. In the degradation of sugar-beet pectin, hydrolysis is affected by the addition of an endopectin lyase but is not influenced by the presence of other accessory enzymes *(49,82)*.

A. niger CinnAE (AnFaeB) has been shown to release <1% of the ferulic groups from sugar-beet pulp. With the addition of a mixture of endo-arabinanase and α-L-arabinofuranosidase, the release of free ferulic acid was increased to 12%. This shows a synergistic effect exerted by the arabinanase and arabinofuranosidase, because these two enzymes alone account for only 5.7% of the release of ferulic acid *(83)*.

Primary Structures of Feruloyl Esterases

Multiple sequence alignments of known feruloyl esterase amino acid sequences suggest that they can be subdivided by sequence similarities with lipase, acetylxylan esterase, chlorogenate esterase/tannase, and xylanase. These four subclasses, FAE types A, B, C, and D, are also distinguished by their substrate specificity, as described earlier.

The FAE-A sequences of *A. niger*, *A. tubingensis*, and *A. awamori* are known (Swiss-Prot: O42807, O42815, and Q9P979, respectively). The *A. niger* and *A. tubingensis* enzymes contain 281 and 280 amino acids, respectively *(49)*. The N-terminal 21 amino acid sequence is characteristic of a typical signal peptide in fungal proteins. The two enzymes show 92 and 96% sequence

identity and similarity in the alignment of their sequences, respectively. The AnFaeA enzymes are very acidic proteins, with a p*I* of 3.3. The calculated molecular mass is 28 kDa for both proteins, but sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) indicates a molecular mass of 36 kDa, suggesting that both enzymes are glycosylated. The Asn100 residue is likely the putative glycosylation site. The *A. awamori* FaeA consists of 281 amino acids, showing 91 and 96% sequence identity and similarity to that of *A. niger*, respectively *(52)*. The enzyme is a glycoprotein 35 kDa with a p*I* of 3.8. Differing from *A. niger* and *A. tubingensis* enzymes, the N-terminal amino acid of *A. awamori* FaeA is pyroglutamate. The three feruloyl esterases show approx 36% identity and approx 50% similarity with the sequences of several lipases, including the *Rhizomucor miedhei* and *Humicola lanuginosa* lipases, and share a short sequence (VTGHSLG) reminiscent of the serine esterase active-site motif (GXSXG).

The amino acid sequences of the FAE type B enzymes are available from *N. crassa* (Q9HGR3) and *P. funiculosum* (Q9HE18). The *N. crassa* FAE-B protein consists of 292 amino acids, including a signal peptide of 18 amino acids *(42)*. The molecular mass estimated by SDS-PAGE is 6 kDa higher than the calculated 29,286 Da for the mature protein. N-glycosylation has been confirmed by electrospray ionization-mass spectrometry, showing a main mass of 35,040 Da. The *P. funiculosum* FAE-B comprises 353 amino acids, including an 18-residue signal peptide. The enzyme has a modular structure, consisting of an N-terminal catalytic domain (276 residues) and a C-terminal carbohydrate-binding domain (39 residues) specific for cellulose binding *(75)*. The two domains are linked by a stretch of 20 amino acids rich in Thr/Ser/Pro. The sequences of the two enzymes show 50% identity and 69% similarity and contain a $GXS_{118}XG$ motif. These type B enzymes share approx 60% sequence similarity with acetyl xylan esterases from *A. ficuum*, *A. awamori*, *A. oryzae*, and *P. purpurogenum*.

The *T. stipitatus* FAE-C (Q70Y21) and AnFaeB (Q8WZI8) are included in the type C subclass according to the recent classification *(43)*. AnFaeB contains 521 amino acids, with a 17-residue signal peptide *(5)*. The mature protein has a calculated p*I* of 4.89 and a calculated molecular mass of 55,551 Da. The enzyme is glycosylated, yielding a molecular mass of 74 kDa for the intact FAE-B, and 60 kDa for the deglycosylated protein, as analyzed by SDS-PAGE. The *T. stipitatus* FAE-C comprises 530 amino acids, with a 25-residue signal peptide and a calculated molecular mass of 55,340 Da *(84,85)*. The protein sequence contains a motif ($GCS_{166}TG$) characteristic of the serine esterase family. The two enzymes share 48% identity and 66% similarity in sequence comparison. Both show ~25 and 40% sequence identity and similarity to chlorogenic acid esterases (*Burkholderia mallei*, *Acinetobacter* sp.) and tannases (*Xanthomonas campestris*, *A. oryzae*), respectively.

The sequences of FAE type D enzymes from *P. equi* (Q9Y871) and *C. japonicus* (Q51815) have been reported. The *P. equi* EstA consists of 536 amino acids with a calculated molecular mass of 55,540 Da. It has a modular structure comprising three domains *(64)*. The N-terminal

domain (40 amino acids) is similar to the docking domain, suggesting that the enzyme forms part of a cellulosome-like multienzyme complex. The C-terminal 270-residue catalytic domain is connected to the docking domain by a linker comprising tandem repeats of a 13-residue motif. The *C. japonicus* EstD has a molecular mass of 58.5 kDa and a modular structure consisting of an N-terminal 273-residue CBD and a C-terminal catalytic domain. The amino acid sequence of the N-terminal domain is identical to the corresponding CBDs of xylanase B and arabinofuranosidase obtained from the same microorganism. The *P. funiculosum* FAE-A, reported only as a nucleotide sequence in the EMBL/GenBank, is a glycoprotein consisting of 345 amino acids with a calculated molecular mass of 36,181 Da. The N-terminal 20 amino acids constitute a potential signal peptide. Its sequence shares 46% identity and 62% similarity with that of *C. japonicus* EstD.

The sequences of feruloyl esterases from several anaerobic microorganisms have been described. The partial sequence of *Orpinomyces* FaeA (Q9P8Y0) is 30% identical to the FAE domains of the bifunctional *C. thermocellum* Xyn10A and 10B, located at the N- and C-terminus, respectively *(56)*. The *B. fibrisolvens* CinI (P70884) and CinII (P94315) share 28% sequence identity. CinI consists of 246 amino acids with a predicted molecular mass of 27,063 Da, and CinII comprises 285 amino acids with a molecular mass of 31,449 Da.

3D Structures of Feruloyl Esterases

The structures of the feruloyl esterases located at the N- and C-terminal domains of *C. thermocellum* xylanase 10A and 10B enzymes have been determined *(86,87)*. More recently, the crystal structure of FAE-A from *A. niger* (AnFaeA) has been published (Fig. 8) *(88)*. The structure of AnFaeA and its mutants in complex with a feruloylated trisaccharide substrate has also been reported *(89,90)*. Both structures display an α/β-fold based on an eight-stranded β sheet surrounded by helices. In FAE_Xyn10A, all eight strands are parallel. In FAE_Xyn10B, the central six strands are parallel, with a pair of antiparallel strands at the C-terminal side of the fold. AnFaeA consists of a central nine-stranded mixed β sheet, two minor two-stranded β sheet arrangements, and seven helices. The β sheet exhibits a superhelical twist, creating approx a 90° angle between the first and the last strands of the protein. The active site contains a catalytic triad (Ser-His-Asp) characteristic of serine protease and esterase families, with the Ser residue located in a conserved pentapeptide having a consensus sequence GXSXG *(91,92)*. The catalytic triad consists of Ser172-His260-Asp230 in FAE_Xyn10A, Ser954-His1058-Asp1018 in FAE_Xyn10B, and Ser133-His247-Asp194 in AnFaeA. To stabilize the carbonyl oxygen anion developed in the tetrahedral intermediate, all three structures contain oxyanion-binding sites ("oxyanion hole"). In FAE_Xyn10A, the NH groups of Met173 and Ile90 are involved in stabilizing interactions. In FAE-Xyn10B structure, the main chain NH groups of Gly866 and Met955 form hydrogen bonds with the carbonyl oxygen. The oxyanion-binding site in AnFaeA involves the backbone NH groups of Thr68 and Leu134.

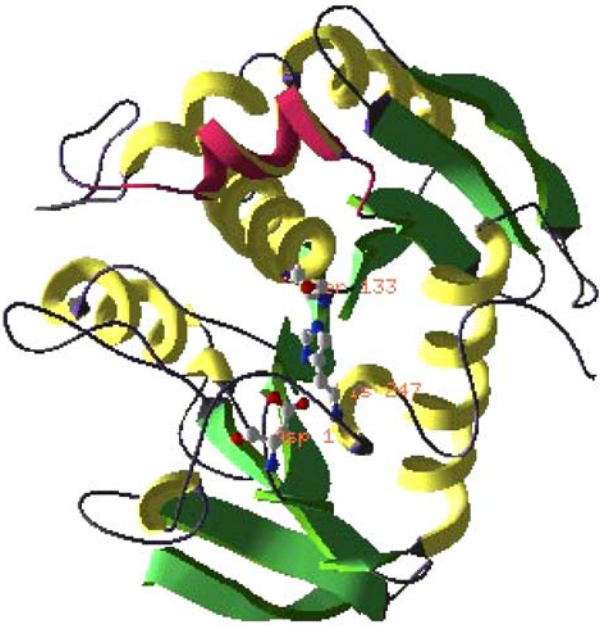


Fig. 8. 3D structure of *A. niger* AnFaeA (PDB 1USW). The helical "lid" is the second helix at the top left.

The ferulic acid binding pocket of these enzymes is located in a long and narrow cavity on the molecular surface created by a number of side chains for the recognition of the aromatic ring substitutions and the sugar part of the substrate. The active sites do not have the same recognition requirements among the three esterases. In FAE_Xyn10A, the nature of interaction is largely hydrophobic, whereas in FAE Xyn10B and AnFaeA, there are side-chain or solvent-mediated interactions with either the polar substituents of the ferulic acid aromatic ring or the second ferulate moiety. In AnFaeA, the ferulic acid interacts through the OH group at C4 and the OCH3 group at C3 with the Tyr80-OH in the AnFaeA-substrate complex. Replacement of Tyr-80 by nonaromatic amino acids causes a reduction in the activity *(90)*.

In the structure of AnFaeA, the substrate-binding site is uniquely confined by a flap (residues 68–80) that displays a helical conformation very similar to that found in the open conformation of the "lid" in a number of microbial lipases. This "lid" structure controls the entry of the substrate to the active site *(93,94)*. When the lipase is adsorbed at the lipid-water interface, the lid undergoes conformational changes from a closed to an open state, making the enzyme-active site accessible to the nonpolar substrate. However, AnFaeA differs from lipases in that the "lid" is more hydrophilic and therefore always exists in the open form. The open conformation is further stabilized by a glycan moiety at Asn79, which extends over the surface. This flap region is not revealed in the structures of FAE_Xyn10A and FAE_Xyn10B.

AnFaeA does not exhibit lipase activity, although there are sequence and structure similarities between the two enzymes *(95)*. Andersen et al. *(96)* engineered FAE activity into *Thermomyces lanuginosa* lipase by site-directed mutagenesis. Lipase mutants containing F113Y substitution at the substrate-binding site exhibited significant FAE activity. Additional mutations of amino acids 83–95 involved in lid opening also increased the activity. In addition to lipases, the same group of researchers also tested cutinases from *A. oryzae*, *Fusarium solani pisi*, and *Humicola insolens* and found low but significant FAE activity in these enzymes.

Reaction Mechanism

Feruloyl esterase displays the classic constellation of the Ser-His-Asp triad, and, thus, the FAE-catalyzed reaction is reasonably very similar to the hydrolytic action of serine proteases, lipases, and other esterases, involving a covalent acylenzyme intermediate *(94)*. In the acylation step, nucleophilic attack by the catalytic Ser-OH on the carbonyl carbon of the scissile bond of the substrate yields a covalent tetrahedral intermediate. This step is facilitated by the catalytic His in a general base catalysis. A collapse of the intermediate to an acylenzyme follows with His-catalyzed protonation of the ester oxygen, liberating the carbohydrate moiety as the product. In the deacylation step, nucleophilic attack by an H_2O molecule assisted by general base catalysis involving the His leads to the formation of a second tetrahedral intermediate. Subsequent His-catalyzed protonation of the Ser-O_g causes breakdown of the intermediate, liberating ferulic acid as the product (Fig. 9).

In the tetrahedral intermediate, the substrate carbonyl oxygen anion is stabilized by the formation of hydrogen bonds with two main chain NH groups in the "oxyanion anion hole," as described in the previous section. The total contribution of hydrogen bonding to the stabilization of charge distribution and energy reduction can reach approx 14 kcal/mol for serine proteases *(97–99)*.

Substrate Specificity

AnFaeA prefers a methoxy group at C-3 of the phenolic ring, since a hydroxy group at C-3 precludes catalysis and an absence of substitution reduces the rate of hydrolysis. AnFaeB prefers no substitutions at C-5 of the phenolic ring, since a methoxy group at C-5 precludes catalysis *(47)*. A methoxy group at C-3 also precludes activity of AnFaeB. In general, increasing the number of methoxy substitutions increases the activity of AnFaeA and decreases the activity of AnFaeB *(31,48,50)*. AnFaeA has a particularly high specificity for substrates with 3-methoxy at C-3 on the aromatic ring. The k_{cat} values for MSA and MFA (both containing a methoxy group at C3) have been determined to be 85 and 71 mol/s, respectively, compared with 0.73 mol/s for M*p*CA, and no activity for MCA *(90)*.

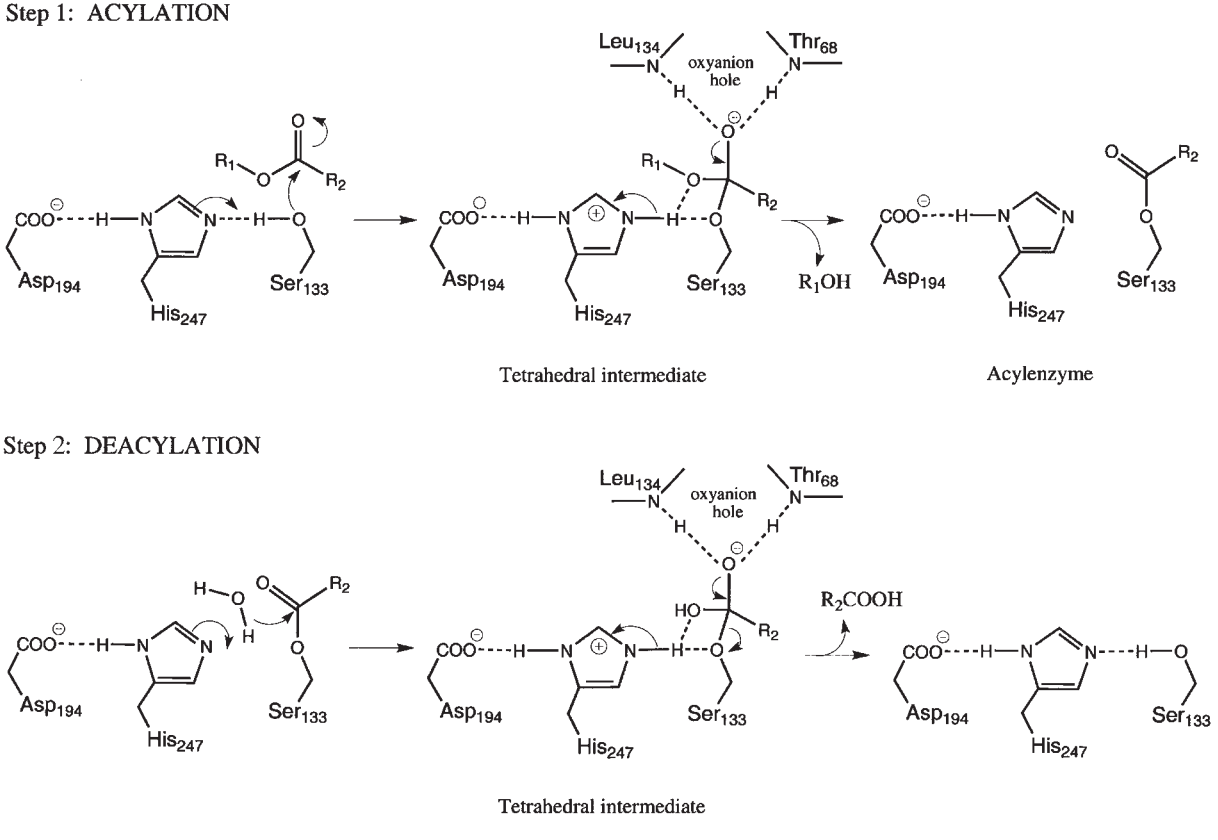


Fig. 9. The proposed mechanism of feruloyl esterases involves the formation and subsequent breakdown of an acylenzyme via a tetrahedral intermediate. (R_1 = carbohydrate moiety; R_2 = phenolic acid moiety). Numbering of the catalytic triad and oxyanion hole residues is based on *A. niger* AnFaeA.

The active sites of feruloyl esterases from mesophilic and thermophilic sources have been probed using methyl phenyalkanoates *(100)*. Type A esterase from the mesophilic fungus *Fusarium oxysporum* (FoFaeA) exhibits a preference for substrates with methoxy substitutions, in contrast to FoFaeB and StFaeB (from *Sporotrichum thermophile*), which are more active toward hydroxylated substrates. The distance between the aromatic ring and the ester bond is a determinant factor for the catalytic activity. The type C esterase StFaeC shows a general preference for substrates containing both methoxy and hydroxyl groups, with the strict requirement of the C4 position being hydroxylated.

Applications of Feruloyl Esterases and Ferulic Acids

In biomass degradation, FAE is an integral part of an enzyme system that acts collectively and synergistically with a variety of other cellulolytic and xylanolytic enzymes to increase the total yield of sugars. The enzyme could be utilized in the enhancement of biomass degradation by disrupting the tight network of lignocelluloses. Untangling the crosslinks among various carbohydrate polymers increases accessibility of the cellulose and hemicellulose fractions to enzymatic hydrolysis. This can potentially increase the yield of hexose and pentose sugar in the bioconversion as feedstock for yeast fermentation to biofuel or other value-added chemicals. The enzyme also aids in solubilizing lignin-polysaccharide complexes in paper pulp processing. The enzyme, together with a number of glycanases and oxidases, has been implicated in the improvement of bread-making quality and related cereal processing *(101)*. The importance of FAE also relates to the enzyme product ferulic acid and feruloylated oligosaccharides, which have potential applications for food and medicine uses. Ferulic acid and its derivatives are strong antioxidants *(102,103)* and have gel-forming properties *(104)*. The biotransformation of ferulic acid to vanillin has been extensively investigated *(105–107)*. The antioxidative and gelling effect has been utilized to form potential protective agents against photooxidative skin damage and for wound management *(108,109)*.

Conclusion

This review has focused on the enzymology of feruloyl esterase. Feruloyl esterase substrates, in monomeric, dimeric, and trimeric forms, are present abundantly in many plants and are associated with cell wall polymers by esterified or etherified crosslinks. FAE enzymes that have been isolated from a number of microorganisms display diverse specificity for synthetic methyl hydroxy-cinnamate substrates as well as plant cell wall–derived feruloylated oligosaccharides. These enzymes, although varying in their sequences, are true serine esterases, all containing the characteristic Ser-His-Asp catalytic triad with a catalytic mechanism involving acylenzyme intermediate formation. The very few sequences and 3D structures available for these enzymes are all derived from microbial sources.

The exploitation of ferulic acid in the development of value-added products for food and medicine uses ultimately depends on a thorough understanding of the fundamentals of the structure/mechanism of these enzymes.

Acknowledgment

Reference to a company and/or products is only for purposes of information and does not imply approval of recommendation of the product to the exclusion of others that may also be suitable. All programs and services of the US Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

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