Esterase Activity Able To Hydrolyze Dietary Antioxidant Hydroxycinnamates Is Distributed along the Intestine of Mammals

Mette F. Andreasen,[†,‡] Paul A. Kroon,[†] Gary Williamson,[†] and Maria-Teresa Garcia-Conesa*[,†]

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom, and Department of Horticulture, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

Hydroxycinnamic acids are effective antioxidants and are abundant components of plant cell walls, especially in cereal bran. For example, wheat and rye brans are rich sources of the hydroxycinnamates ferulic acid, sinapic acid, and *p*-coumaric acid. These phenolics are part of human and animal diets and may contribute to the beneficial effects derived from consumption of cereal bran. However, these compounds are ester linked to the main polymers in the plant cell wall and cannot be absorbed in this complex form. The present work shows that esterases with activity toward esters of the major dietary hydroxycinnamates are distributed throughout the intestinal tract of mammals. In rats, the cinnamoyl esterase activity in the small intestine is derived mainly from the mucosa, whereas in the large intestine the esterase activity was found predominantly in the luminal microflora. Mucosa cell-free extracts obtained from human duodenum, jejunum, and ileum efficiently hydrolyzed various hydroxycinnamoyl esters, providing the first evidence of human cinnamoyl esterase(s). This study first demonstrates the release by human colonic esterase(s) (mostly of microbial origin) of sinapic acid and *p*-coumaric acid from rye and wheat brans. Hydrolysis by intestinal esterase(s) is very likely the major route for release of antioxidant hydroxycinnamic acids in vivo.

Keywords: *Sinapic acid; ferulic acid; p-coumaric acid; caffeic acid; chlorogenic acid; hydroxycinnamates; wheat; rye; bran; intestinal esterases; rat; human*

INTRODUCTION

Epidemiological studies suggest a link between the consumption of whole grain products and the prevention of chronic diseases such as coronary heart disease and certain forms of cancer, particularly those associated with the alimentary tract (e.g., colorectal cancer) and hormone-related cancers (e.g., breast cancer) (*1*−*3*). The beneficial health effects derived from the intake of a diet rich in whole grain cereals has often been attributed to dietary fiber (*1*) or to some of the components associated with the fiber, that is, lignans and phenolic acids (*4*). Cereal brans contain significant quantities of the phenolic compounds hydroxycinnamic acids (*5*, *6*). Hydroxycinnamic acids exhibit in vitro chemoprotective and antioxidant properties (*7*, *8*), and it is suspected that they may contribute toward the beneficial effects of a bran-rich diet (*1*).

The major hydroxycinnamic acids present in cereals are ferulic acid, *p*-coumaric acid, and sinapic acid, with ferulic acid being the most abundant (*9*−*11*). Sinapic acid, which is itself a potent antioxidant (*12*), is present in significant quantities in the bran of rye kernels [410 μg g^{-1} of rye bran dry matter (dm)] (*6*). Caffeic acid has been also detected in small amounts in rye (<20 μg g^{-1}) and oat (*9*). Esterified sinapate and caffeate are present in small quantities in aqueous soluble fractions prepared from several classes of Canadian wheat and wheat flours. Sinapic acid was, however, not detected in any of the insoluble fractions obtained from the same wheat varieties in which the major constituent was ferulic acid (*13*). Regular consumption of cereal bran and bran-enriched products may therefore result in ingestion of significant amounts of hydroxycinnamates. For example, an intake of 10 g of rye bran would result in ingestion of ∼40 mg of esterified ferulic acid, 4 mg of sinapic acid, and 2 mg of *p*-coumaric acid (*6*).

The potential health benefits of the hydroxycinnamic acids and derivatives have mostly been related to their effective antioxidant capacity. For example, the ability of these compounds to protect low-density lipoprotein (LDL) from oxidative modifications has been reported by several authors (*12*, *14*). It is believed that oxidized LDL plays a key role in the pathogenesis of atherosclerosis, leading to plaque buildup in arteries and consequently coronary heart disease (*15*, *16*). The ability of hydroxycinnamic acids to inhibit oxidation of human LDL decreased in the following order: caffeic acid > sinapic acid ≫ ferulic acid > *p*-coumaric acid (*8*, *12*, *14*). The chemical structures of these hydroxycinnamic acids are shown in Figure 1. In addition, the hydroxycinnamates also exhibit inhibitory effects on tumor promotion (*17*, *18*) and can block the formation of mutagenic compounds such as nitrosamines (*19*).

To fully understand the implications of dietary hydroxycinnamates in human and animal health, it is necessary to determine the mechanisms by which these compounds become bioavailable and may exert their effects in vivo. Almost all hydroxycinnamates present

* Author to whom correspondence should be addressed [telephone +44 (0)1603 255047; fax + 44 (0)1603 255038; e-mail maria.conesa@bbsrc.ac.uk].

[†] Institute of Food Research.

[‡] Danish Institute of Agricultural Sciences.

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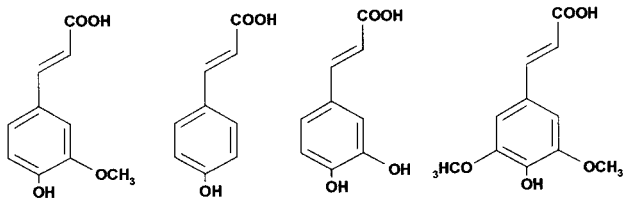

Figure 1. Chemical structures of some dietary monomeric hydroxycinnamates.

in dietary cereals (ferulic acid, sinapic acid, and *p*-coumaric acid) are ester-linked to plant cell wall polymers and cannot be absorbed in this complex form. A notable exception is caffeic acid, which is present in most foods as part of chlorogenic acid, a low molecular weight, soluble ester of caffeic acid and quinic acid, which is found at high concentrations in coffee and fruits (*20*). Enzymes able to break the ester bonds and release free phenolic acids are therefore required as the first step in the uptake and metabolism of dietary hydroxycinnamates. The presence of such esterase activity has been demonstrated in the large intestine (microflora enzymes) of rats and humans (*21*−*23*). The esterase(s) were able to hydrolyze and release the hydroxycinnamates ferulate and *p*-coumarate, which then became available for absorption into the circulatory system (*23*−*26*). An esterase activity able to hydrolyze chlorogenic acid has been demonstrated in human colonic microflora (*27*). Chlorogenic and caffeic acid can be absorbed from the small intestine into the circulatory system of humans (*20*), whereas in rats only caffeic acid can be absorbed (*28*). To date, there are no reports on the bioavailability of sinapic acid.

Because intestinal esterases are very likely the major route for the release of hydroxycinnamic acids in vivo, then the site, levels, and specificity of these enzymes are critical factors influencing the bioavailability of these phenolic antioxidants. In this paper we investigated the distribution of cinnamoyl esterase activity in the intestine of rats and humans, using synthetic hydroxycinnamoyl esters as model substrates. Furthermore, as the antioxidant hydroxycinnamates are abundant in cereals, we also determined the release of these phenolics from dietary fiber (rye bran and wheat bran).

MATERIALS AND METHODS

Substrates and Chemicals. Methyl ferulate (MFA), methyl sinapate (MSA), methyl caffeate (MCA), and methyl *p*-coumarate (M*p*CA) were purchased from Apin Chemicals Ltd. 5-*O*-(*trans*-Feruloyl)-L-Ara*f* (AF) was isolated from maize bran by acid hydrolysis and gel filtration chromatography (*29*). Chlorogenic acid was obtained from ICN Biomedicals Inc. *trans*-Ferulic acid, *trans*-sinapic acid, *trans*-caffeic acid, *trans*-*p*-coumaric acid, and *trans*-cinnamic acid were obtained from Sigma Chemical Co. Destarched wheat bran (DSWB) was obtained from Agro-industries Research et Dévelopement (ARD), Pomacle, France. Rye bran (13% of the grain) was made from whole rye grain (cv. Esprit, harvested in 1997), milled on a laboratory mill (Brabender, Quadrumat Junior) and passed through a 0.71 μm sieve. The final rye bran product was sterilized at 100 °C in 0.05 M trifluoroacetic acid for 1 h. All other chemicals were of analytical or HPLC grade purity.

Preparation of Intestinal Extracts. Homogenate extracts from rat and human intestine were prepared as described previously (*30*).

Intestinal Esterase Activity on Hydroxycinnamate Esters. Intestinal cinnamoyl esterase activity was determined using a range of hydroxycinnamates as model substrates. Activity was assayed by incubating the substrates (MFA, MCA, MSA, M*p*CA, and chlorogenic acid; final concentration = 1 mM, final volume = 0.5 mL; AF, final concentration = 0.1 mM, final volume = 0.5 mL) with the intestinal mucosa extract or gut content extract for 1 h in PBS buffer (pH 7.0) at 37 °C. The procedure is a slightly modified version of the method described by Faulds and Williamson (*31*). An amount of enzyme extract was used that gave <5% conversion of substrate to product and was in the linear part of the reaction curve. Reactions were terminated by the addition of glacial acetic acid (0.2 mL, final pH <2.5). Controls containing the reaction mixture plus glacial acetic acid (0.2 mL) were also incubated to test for the presence of background peaks. Samples and blanks were centrifuged at 13000*g* for 10 min and filtered (0.2 μm) prior to HPLC-DAD analysis of the released hydroxycinnamic acids.

Preparation of a Soluble Fraction from Cereal Bran. Low molecular mass material containing esterified hydroxycinnamates was solubilized by enzymic digestion of wheat bran and rye bran using a xylanase from *Trichoderma viride* (Novozym 431 L, Novo Nordisk A/S) (*30*).

Total Alkali-Extractable Hydroxycinnamates. To determine total esterified hydroxycinnamates in the cereal bran and in the solubilized fraction, NaOH (1.0 M final concentration) was added to 200 mg of the bran or 4.0 mL of the soluble fraction (final volume of 10 mL), followed by incubation for 18 h at room temperature with stirring under nitrogen and in the absence of light. Reactions were terminated by the addition of HCl (2.0 M, final pH <2), and *trans*-cinnamic acid (25 μg) was added as internal standard. Acidified samples were extracted with ethyl acetate (3 × 5 mL). The combined ethyl acetate fractions were dried through anhydrous Na_2SO_4 and evaporated under reduced pressure at 30 °C. The dry residue was first dissolved in 1 mL of methanol, then diluted with 1 mL of Milli-Q water (final pH 2.0), and filtered (0.2 μm) prior to HPLC-DAD analysis of the released hydroxycinnamic acids.

Release of Esterified Hydroxycinnamic Acids from Cereal Bran and a Soluble Fraction by Intestinal Esterases. Samples [200 mg of cereal bran in 4 mL of PBS (pH 7.0) or 4.0 mL of soluble fraction (pH 7.0)] were incubated with human fecal extract (4.0 mL, pH 7.0) for 18 h at 37 °C. Blanks containing the cereal bran or the soluble material in buffer were incubated as controls. A second blank containing human fecal extract in buffer was also incubated to check for the presence of hydroxycinnamates in the fecal sample. Reactions were terminated by the addition of HCl (2.0 M, pH <2). *trans*-Cinnamic acid (10 μg) was added as internal standard, and the reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined ethyl acetate fractions were dried with anhydrous Na_2SO_4 and evaporated under reduced pressure at 30 °C. The dry residue was dissolved in 0.5 mL of methanol, then diluted with 0.5 mL of Milli-Q water (final pH 2), and filtered (0.2 μm) prior to HPLC-DAD analysis of the released hydroxycinnamic acids.

Analysis of Hydroxycinnamic Acids by HPLC-DAD. Hydroxycinnamic acids (ferulic, caffeic, *p*-coumaric, and sinapic acid) were analyzed by reversed-phase HPLC-DAD (*30*).

RESULTS

Distribution of Cinnamoyl Esterase Activity on Soluble Substrates in the Intestine of Rats and Humans. The distribution of cinnamoyl esterase activity in the intestine of rats was determined using the methyl esters of ferulic acid, *p*-coumaric acid, caffeic acid, and sinapic acid as model substrates (Figure 2). Under the conditions of our assay, mucosal extracts from all three sections of small intestine were active on all of the methyl esters and, for any particular substrate, the levels of activity (specific activities) were similar for extracts from duodenum, jejunum, and ileum. However, the level of esterase activity was markedly lower in the

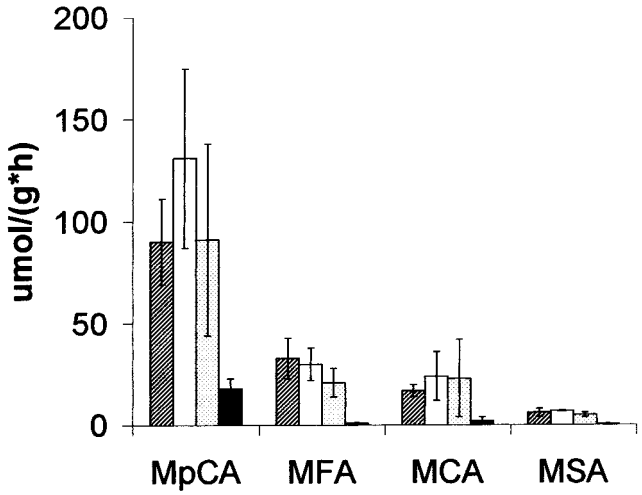

Figure 2. Cinnamoyl esterase activity in rat intestinal mucosa: (▨) DM, duodenum mucosa; (□) JM, jejunum mucosa; (▩) IM, ileum mucosa; (■) LM, large intestine mucosa. Methyl esters (MFA, MCA, MSA, and M*p*CA, 1 mM) were incubated with intestinal mucosa for 1 h at pH 7.0 and 37 °C. Rates (micromoles of product released per gram of mucosa per hour) were measured using an amount of intestinal mucosa that gave <5% conversion of substrate to product and was in the linear part of the reaction curve. Data are mean values of rates ± SD ($n = 4$).

mucosal tissue from the large intestine. No differences in substrate specificity were observed between the various sections of intestinal mucosa, which showed the highest activity on M*p*CA (~100 μmol of *p*-coumaric acid g^{-1} h^{-1}). The cinnamoyl esterase activity in rat intestinal mucosa on hydroxycinnamates decreased in the following order: M*p*CA > MFA ≈ MCA > MSA. These results indicate that the presence of substitutions on the phenolic ring of the substrate has an effect on the intestinal cinnamoyl esterase activity and, thus, activity decreases with an increase in the number of substitutions on the benzene ring of the phenolic substrate.

The cinnamoyl esterase activity was also measured in the gut contents from each of the small and large intestine sections. To compare lumen esterase activity with mucosa activity, values presented in Table 1 are expressed as total esterase activity. Our studies show that most of the cinnamoyl esterase activity measured in the small intestine of rat was located principally in the mucosa. Esterase activity in the small intestine lumen accounted for only 5−10% of the total activity in this part of the intestine. However, in the large intestine, cinnamoyl esterase activity was found predominantly in the lumen (50−95%). Additionally, we examined the ability of the rat intestine samples to hydrolyze feruloylated arabinose and chlorogenic acid. Tissue extracts from both small and large intestine of rats are capable of efficiently hydrolyzing feruloylated arabinose. No activity on chlorogenic acid was detected in any of the mucosa preparations from small and large intestine or in the small intestine lumen. However, some release of caffeic acid was observed when chlorogenic acid was incubated with the large intestine contents.

We next investigated the ability of human small intestine mucosa and human fecal cell-free extracts to hydrolyze hydroxycinnamates using MFA as a model substrate. Human mucosa extracts from all three sections of small intestine exhibited esterase activity on MFA: 460 ± 150 nmol g^{-1} h^{-1} ($n = 3$) in the duodenum, 670 ± 290 nmol g^{-1} h^{-1} ($n = 3$) in the jejunum, and 237 nmol g^{-1} h^{-1} ($n = 1$) in the ileum. Human fecal cell-free extracts from healthy volunteers also exhibited esterase activity on MFA, and this was ~10-fold higher (3200 ± 1800 nmol g^{-1} h^{-1}, $n = 4$) than the activity detected in any of the small intestine samples.

Release of Hydroxycinnamates from Cereal Bran by Human Fecal Esterases. We investigated whether human fecal esterases were able to specifically release some of the hydroxycinnamates from dietary materials, namely, wheat and rye brans. Composition in saponifiable hydroxycinnamates of the wheat bran and rye bran samples used in this study is included in Table 2. The major phenolic acid present is ferulic acid followed by *p*-coumaric and sinapic acids. It is interesting to note that wheat bran contains ~75 μg/g of esterified sinapic acid; this has not been published earlier in the literature. Samples of wheat bran or rye bran were also incubated with human fecal extracts. Under the conditions of our assay, the esterase(s) present in the human fecal extract released ferulic acid, *p*-coumaric acid, and sinapic acid from both types of bran (Table 2). The fecal esterase(s) released ~20% of each of the hydroxycinnamic acids from wheat bran. Under the same conditions, these enzymes released 18% of ferulic acid, 10% of *p*-coumaric acid, and 53% of sinapic acid from rye bran. Human colon microflora esterases are more efficient at releasing sinapic acid from rye bran than from wheat bran. This is the first report on the release by human fecal esterase(s) of sinapic acid and *p*-coumaric acid from dietary cereal brans.

We used an esterase-free xylanase from *Trichoderma viride* to solubilize a proportion of the esterified hydroxycinnamates from wheat bran and rye bran. Following alkaline hydrolysis of the soluble fraction, we detected and quantified ferulic, *p*-coumaric, and sinapic acid (Table 2). Solubilization by the xylanase (expressed as a proportion of the total amount of compound present in the bran) of ferulic, *p*-coumaric, and sinapic acid was 67, 45, and 9% respectively, for wheat bran and 73, 23, and 46% for rye bran. The ability of colon microflora esterase(s) to release esterified hydroxycinnamic acids from the bran solubilized fraction was assessed by incubating samples of the soluble fraction obtained from wheat bran or rye bran with the fecal extracts. Human fecal esterases were able to release hydroxycinnamic acids from both soluble fractions (Table 2). The fecal esterase(s) released 100, 80, and 38% of ferulic, *p*-coumaric, and sinapic acids, respectively, from soluble wheat bran and 64, 13, and 53% of ferulic, *p*-coumaric, and sinapic acids, respectively, from soluble rye bran. Human fecal esterases were less efficient at releasing ferulic acid and *p*-coumaric acid from soluble rye bran than from soluble wheat bran. However, the release of sinapic acid was slightly more efficient from the rye bran. The values of released *p*-coumaric acid are an underestimation of the real values because some of this hydroxycinnamic acid is further metabolized. In vitro solubilization of the bran with an esterase-free fungal xylanase provides a soluble substrate to which the microbial esterases have better access as shown by a higher percentage of released esterified hydroxycinnamates from these soluble substrates.

DISCUSSION

Most hydroxycinnamic acids are effective antioxidants in vitro (*7*, *8*, *12*, *14*), and as part of the diet they may play a role in disease prevention. They are abundant

Table 1. Distribution of Cinnamoyl Esterase Activity in Rat Intestine[a]

source of enzyme		substrate			
		MFA[b] (μmol/h)	MSA[c] (μmol/h)	MCA[c] (μmol/h)	M*p*CA[c] (μmol/h)
mucosa from	duodenum	13 ± 4	3 ± 1	8 ± 3	42 ± 18
	jejunum	45 ± 16	10 ± 2	32 ± 11	170 ± 40
	ileum	47 ± 23	11 ± 2	46 ± 34	190 ± 90
	large intestine	0.3 ± 0.2	0.1 ± 0.05	0.8 ± 0.7	7 ± 3
gut content from	duodenum	0.1 ± 0.1	0.3 ± 0.2	2 ± 2	6 ± 5
	jejunum	2 ± 3	0.4 ± 0.3	7 ± 5	25 ± 10
	ileum	3 ± 4	0.5 ± 0.4	15 ± 19	17 ± 12
	large intestine	2 ± 1	11 ± 11	4 ± 4	6 ± 4

[a] Intestinal mucosa or gut content extracts were incubated in vitro with the methyl esterified hydroxycinnamates (MFA, MSA, MCA, M*p*CA) at pH 7.0 and 37 °C for 1 h. Values are presented as total esterase activity (rate × grams of intestinal sample; micromoles per hour). Data are mean values ± SD. [b] $n = 5$ [c] $n = 4$.

Table 2. Release of Ester-Linked Hydroxycinnamates from Cereal Bran and from a Soluble Fraction by Human Fecal Extract[a]

cereal type	sample	treatment	units	ferulic acid	*p*-coumaric acid	sinapic acid
wheat	bran	NaOH	μg/g	5410 ± 360	169 ± 4	75 ± 4
	bran	human fecal extract	μg/g	1360 ± 140 (25%)[b]	40 ± 2 (24%)	14.9 ± 1.3 (20%)
	soluble fraction	NaOH	μg/mL	361 ± 8	7.6 ± 0.1	0.66 ± 0.02
	soluble fraction	human fecal extract	μg/mL	370 ± 14 (102%)	6.1 ± 1.1 (80%)	0.25 ± 0.12 (38%)
rye	bran	NaOH	μg/g	2780 ± 210	190 ± 10	389 ± 2
	bran	human fecal extract	μg/g	505 ± 6 (18%)	18.7 ± 0.1 (10%)	207 ± 8 (53%)
	soluble fraction	NaOH	μg/mL	203 ± 13	4.4 ± 0.3	17.9 ± 0.9
	soluble fraction	human fecal extract	μg/mL	130 ± 30 (64%)	0.56 ± 0.96 (13%)	9.4 ± 3.0 (53%)

[a] Incubation of cereal bran or a solubilized fraction with human fecal extract was performed at pH 7.0 and 37 °C for 18 h. Values are presented as micrograms of compound released per gram of bran or per milliliter of solution. Data are mean values ± SD ($n = 3$). [b] Percentage released by the human fecal extract relative to the total esterified content in the bran or in the soluble fraction.

in whole grain cereals (*5*, *6*), where they are found predominantly esterified to polysaccharides (*32*). To explain their potential beneficial effects in vivo, it is important to establish the mechanism by which dietary hydroxycinnamates may become bioavailable. The main aim of this work was to investigate the presence, levels, and specificity of cinnamoyl esterase activity in the intestine of rats and humans, which will influence the bioavailability of hydroxycinnamates.

There are some previous reports in the literature on the presence in the digestive tract of humans of esterase(s) with activity on dietary phenolic esters. A catechin esterase activity was described in human saliva, but its possible microbial origin was not completely excluded (*33*). The presence of colonic luminal esterase(s) able to release some of the ester-linked cell wall hydroxycinnamates was reported previously in rats and humans (*21*−*23*), but it was not known whether these esterases were also present in other sections of the intestinal tract and whether they could have an epithelial origin. In 1996, Buchanan and co-workers demonstrated that ferulic and *p*-coumaric acids were released from spinach cell walls and absorbed through the stomach and small intestine of rats, suggesting the presence of esterase activity in these sections of the intestinal tract (*23*), but a full study on the origin, levels, and specificity of this activity was not undertaken. The present study has revealed that esterases with the ability of hydrolyzing hydroxycinnamate esters at appreciable rates are distributed all along the small and large intestine of rats and humans. The cinnamoyl esterase activity is present both in the mucosal cells and in the lumen. The activity measurements were carried out using whole cell extracts as the source of enzyme and, thus, determination of the precise localization of the cinnamoyl esterase(s) within the mucosa cells was not pursued in this work.

Chymotrypsin-like serine esterases have been histochemically demonstrated in the granules of intraepithelial mucosal mast cells of the gastrointestinal tract of rats and humans (*34*). Other cinnamoyl esterases such as FAEA from *Aspergillus niger* have been recognized as serine hydrolases (*35*) and, thus, it is possible that the intraepithelial serine hydrolases found in the mast cells may be responsible for the cinnamoyl esterase activity detected in the small intestine mucosa. Hydroxycinnamates are mostly ester-linked to hemicelluloses, forming large and complex molecules that cannot be absorbed through the mucosal barrier into the epithelial cells. For the mucosal cinnamoyl esterase(s) to contribute to the hydrolysis of plant hydroxycinnamates, they would need to be located on the brush border membrane facing the intestinal lumen. Other esterases such as retinyl ester hydrolases are intrinsic to the brush border membrane of rat and human small intestine (*36*, *37*). Additionally, the cinnamoyl esterases may be released into the lumen when cells are exfoliated.

The cinnamoyl esterase activity detected in the rat small intestine lumen may therefore have an epithelial origin and/or a microbial origin (*38*). Phenolic sugar esters are hydrolyzed by porcine pancreatic esterases (*39*). We detected cinnamoyl esterase activity in cell-free extracts obtained from rat pancreas (data not shown). It is possible that the pancreatic secretion may contribute to the cinnamoyl esterase activity detected in the small intestine lumen. The results presented in this work, together with those reported by Buchanan and co-workers (*23*), indicate that a proportion of the ester bonds which link hydroxycinnamic acids to the plant cell wall polysaccharides may already be cleaved in the small intestine and, thus, some antioxidant hydroxycinnamic acids may be available for absorption in the small intestine.

Our study has shown that colonic microflora esterases are capable of releasing free ferulic, sinapic, and *p*-coumaric acid from wheat and rye brans. The hydrolysis of hydroxycinnamates in the large intestine may be enhanced by other enzymes (e.g., xylanases) that are capable of digesting the plant cell wall polymers to form small soluble cinnamoyl oligosaccharides to which esterases have better access (*21*). A common side chain of grass cell wall arabinoxylans, 2-*O*-*β*-D-xylopyranosyl-(5-*O*-feruloyl)-L-arabinose, is rapidly hydrolyzed by bacteria present in the rat cecum (*22*). The release and absorption of hydroxycinnamates in the intestine from certain processed food (e.g.. bread) may be enhanced. In food processing such as bread-making the use of hemicellulases may form smaller cinnamoyl esters, which can then be hydrolyzed more easily by the intestine esterase(s), thus increasing the bioavailability of hydroxycinnamates.

An esterase activity on chlorogenic acid was detected in human fecal extracts, but no activity was detected in extracts prepared from human small intestine (*27*). We found similar results in the rat intestinal tract. It appears that only colonic microbial esterases are able to specifically hydrolyze chlorogenic acid. Mucosal esterase(s) cannot hydrolyze caffeoylquinic esters (chlorogenic acid) but are capable of cleaving a caffeoyl methyl ester.

In conclusion, we have demonstrated, using in vitro assays, that (1) cinnamoyl esterases able to hydrolyze hydroxycinnamates at appreciable rates are distributed throughout the intestinal tract of rats and humans. In the small intestine, the activity is located mainly in the mucosa cells, whereas in the large intestine most of the esterases are of microbial origin. Human microbial esterases (2) are able to release dietary ester-linked hydroxycinnamates from dietary fiber. This study first shows the release of *p*-coumaric and sinapic acid from cereal bran. Hydrolysis by intestinal esterase(s) is very likely the major route for release of antioxidant hydroxycinnamic acids in vivo. The released hydroxycinnamic acids become then available for absorption and have the potential to exert some effects on health.

ABBREVIATIONS USED

MCA, methyl caffeate; MFA, methyl ferulate; M*p*CA, methyl *p*-coumarate; MSA, methyl sinapate, AF, 5-*O*-(*trans*-feruloyl)-L-Ara*f*.

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