GLUCURONIDATION AND SULFATION OF THE TEA FLAVONOID (−)-EPICATECHIN BY THE HUMAN AND RAT ENZYMES

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(−)-Epicatechin (EC) is one of the flavonoids present in green tea, suggested to have chemopreventive properties in cancer. However, its bioavailability is not clearly understood. In the present study, we determined the metabolism of EC, focusing on its glucuronic acid and sulfate conjugation using human liver and intestinal microsomes and cytosol as well as recombinant UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) isoforms in comparison with that occurring in the rat. Surprisingly, EC was not glucuronidated by the human liver and small intestinal microsomes. There was also no evidence of glucuronidation by human colon microsomes or by recombinant UGT1A7, which is not present in the liver or intestine. Interestingly, in the rat liver microsomes EC was efficiently glucuronidated with the formation of two glucuronides. In contrast, the human liver cytosol efficiently sulfated EC mainly through the SULT1A1 isoform. For the intestine, both SULT1A1 and SULT1A3 contributed. Other SULT isoforms contributed little. High-performance liquid chromatography of the sulfate conjugates showed one major sulfatase-sensitive peak with all tissues. An additional minor sulfatase-resistant peak was formed by the liver and intestinal cytosol as well as with SULT1A1 but not by the Caco-2 cytosol and SULT1A3. In the rat, EC sulfation was considerably less efficient than in the human liver. These results indicate that sulfation is the major pathway in EC metabolism in the human liver and intestine with no glucuronidation occurring. There was also a large species difference both in glucuronidation and sulfation of EC between rats and humans.

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There is a great interest in the role of tea for maintenance of health and in the treatment of disease. (−)-Epicatechin (EC1), (−)-epicatechin-3-gallate, (−)-epigallocatechin-3-gallate, and (−)-epigallocatechin are the main flavonoids found in tea thought to be responsible for these beneficial effects. Support for such effects is particularly strong in cancer, both in animals (Stoner and Mukhtar, 1995) and in humans (Katiyar and Mukhtar, 1996). These effects have long been thought to be produced by the potent antioxidant effects (Salah et al., 1995) of the tea flavonoids. Recent studies using a variety of cell culture models demonstrate a number of effects of the tea flavonoids on cell proliferation and apoptosis and specific signal transduction events, including mitogen-activated protein kinase and nuclear fac-

tor-κB (Lin and Lin, 1997; Morre et al., 2000; Chung et al., 2001; Yang et al., 2001).

In view of these observations, it is of significance that the oral bioavailability of the tea flavonoids is poorly understood. Various preparations of tea flavonoids have been administered to humans and their concentrations in plasma determined. From these studies it is clear that the catechins do reach the systemic circulation but that the oral bioavailability is low (Lee et al., 1995; Nakagawa et al., 1997; Yang et al., 1998; Baba et al., 2000; Chow et al., 2001). In the most recent study only 1.68% of the ingested catechins were in human plasma, urine, and feces, and the apparent bioavailability of the gallated catechins were lower than the nongallated forms (Warden et al., 2001). The oral bioavailability of the tea flavonoids has also been shown to be low in rats (Chen et al., 1997; Zhu et al., 2000). Low oral bioavailability could be due to poor absorption in the intestine and/or extensive metabolism by the intestine and the liver.

In a previous study, we selected one of the tea catechins [i.e., EC (Fig. 1)], to study enterocyte absorption, using the human intestinal Caco-2 cell line. Interestingly, there was no or very low absorption (Vaidyanathan and Walle, 2001). This was, at least in part, due to the MRP2 efflux transporter. When MRP2 was inhibited by MK-571, absorption of EC could be observed, but it was low. This suggested that another factor (i.e., metabolism) was further limiting. Although metabolism of the tea flavonoids in humans has been indicated to be conjugative, including both glucuronidation and sulfation (Lee et al., 1995; Yang et al., 1998; Baba et al., 2000; Chow et al., 2001), the measurements have been indirect after enzymatic hydrolysis of conjugates, thus, not molecularly specific. In rats on the other hand,
glucuronidation has been well documented to be the most important conjugative pathway (Harada et al., 1999; Okushio et al., 1999).

In the present study, we examined the metabolism of EC by both glucuronidation and sulfation using human liver and intestinal microsomes and cytosols as well as recombinant UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) isoforms. As a comparison, we also examined the metabolism of EC by similar preparations from the rat.

Experimental Procedures

Materials. (−)-Epicatechin (Fig. 1), (+)-catechin, quercetin, uridine 5′-diphosphoglucuronic acid (UDPGA), beef liver β-glucuronidase, sulfatase from *Aerobacter aerogenes*, β-saccharic acid 1,4-lactone, alamethicin, tetrabutylammonium hydrogen sulfate, and trifluoroacetic acid (spectrophotometric grade) were obtained from Sigma-Aldrich (St. Louis, MO). [35S]PAPS (1.0−1.5 Ci/mmol) was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Pooled human liver microsomes and cytosol, pooled human intestinal microsomes, pooled undrugged- and Aroclor 1254-induced rat liver microsomes, and recombinant human UGT1A7 and UGT1A10 were obtained from Gentest Corp. (Woburn, MA). All other chemicals used were of analytical grade.

Tissue Preparations. Human colon and intestinal tissues were obtained from the National Disease Research Interchange (Philadelphia, PA). The colon microsomes were prepared by scraping off the mucosa with a glass slide followed by homogenization with a Teflon/glass homogenizer in 1.5% KCl and differential centrifugation to obtain the 100,000×g supernatant. The pellets were then resuspended in 100 mM phosphate buffer (pH 7.25) containing 10% glycerol, 1 mM EDTA, 20 mM butylated hydroxytoluene, and 100 mM phenylmethylsulfonyl fluoride. The rat liver cytosols from three male Sprague-Dawley rats (33–36 days old; Harlan Sprague Dawley Inc., Indianapolis, IN) were prepared by homogenizing the tissue with a polystyrene homogenizer in 5 mM phosphate buffer (pH 6.5) containing 250 mM sucrose and 3 mM mercaptoethanol followed by centrifugation to obtain the 100,000×g supernatant. Human jejunal cytosol was prepared by scraping off the mucosa with a glass slide followed by homogenization and centrifugation as above to obtain the mucosal 100,000×g supernatant. Human jejunal cytosol was prepared by scraping off the mucosa with a glass slide followed by homogenization and centrifugation as above to obtain the mucosal 100,000×g supernatant. Human jejunal cytosol was prepared by scraping off the mucosa with a glass slide followed by homogenization and centrifugation as above to obtain the mucosal 100,000×g supernatant. Human jejunal cytosol was prepared by scraping off the mucosa with a glass slide followed by homogenization and centrifugation as above to obtain the mucosal 100,000×g supernatant.

Recombinant Sulfotransferases. Recombinant human monoamine-form phenol sulfotransferase, SULT1A3 (Ganguly et al., 1995), human dehydroepiandrosterone sulfotransferase, SULT2A1 (Comer et al., 1993), and human estrogen sulfotransferase, SULT1E1 (Falany et al., 1995) were purified from pkk233−2 constructs obtained from Dr. C. N. Falany and expressed in *Escherichia coli*. Recombinant human histidine-labeled P-form phenol sulfotransferase, SULT1A1 was prepared as described (Lewis et al., 1996). Because of the efficient affinity column cleanup, SULT1A1 was >99% pure; however, the other SULT isoforms contained multiple non-SULT proteins even after ion-exchange chromatography. Further purification of the isoforms was unsuccessful due to limited stability.

Glucuronidation of EC. EC (50–1000 μM) and 10 μl of human intestinal, 25 μl of colon or 10 μl of liver microsomes (200, 400, 200 μg of protein, respectively), 20 μl of recombinant UGT1A7 or UGT1A10 (400 μg of protein) in a final volume of 500 μl of 50 mM Tris HCl buffer (pH 7.8) with 10 mM MgCl₂ were preincubated for 5 min at 37°C. The reactions were initiated by the addition of 1 mM UDPGA. The reaction mixtures were incubated at 37°C for 60 min. The samples were cooled on ice and subjected to solid-phase extraction using Oasis Hydrophilic-Lipophilic Balance 1cc C₁₈ extraction cartridges (Waters Corp., Milford, MA). The cartridges were washed with 1-ml methanol and equilibrated with 1-ml water. After loading 0.5 ml of the sample, the cartridges were washed with 5% methanol and eluted with 2 ml of 100% methanol. The methanol eluate was dried under N₂ gas at 40°C, and the sample was redissolved in 250 μl of mobile phase for HPLC analysis.

In other experiments EC (1−100 μM) and 20 μl of pooled rat liver microsomes or Aroclor-induced rat liver microsomes (400 and 200 μg of protein, respectively) were used and the reactions done as mentioned above.

Sulfation of EC. The sulfation of EC catalyzed by the different sulfotransferase enzyme sources was determined using the previously described ionpair extraction method (Varin et al., 1987). The typical reaction mixture contained 0.1 to 200 μM of EC, 1 μM [35S]PAPS and 2.5 μl of pooled human liver cytosol (50 μg of protein), 2.5 μl of human jejunal cytosol (30 μg), Caco-2 cytosol (225 μg) or 0.25 μl recombinant SULT in 33 mM Tris-HCl buffer, pH 7.4, with 8 mM dithiothreitol and 0.0625% bovine serum albumin in a total volume of 100 μl. The samples were incubated for 30 min at 37°C, and the reactions were terminated by the addition of 10 μl 2.5% acetic acid, 20 μl of 0.1 M tetrabutylammonium hydrogen sulfate and 500 μl of ethyl acetate. After through mixing and centrifugation, 400 μl of the ethyl acetate extract was subjected to liquid scintillation counting after the addition of biodegradable counting scintillant (Amersham Biosciences, Piscataway, NJ).

In another set of experiments, sulfation of EC (5 μM) was carried out as mentioned above, but the ethyl acetate extract was taken to dryness under N₂ gas and reconstituted in mobile phase and analyzed by HPLC (see below). One-minute fractions of the HPLC eluate were collected and analyzed by liquid scintillation counting.

Sulfation of EC (1−100 μM) was also carried out using 2.5 μl of rat liver cytosol (80 μg of protein) as above.

HPLC Analysis. EC and its metabolites were analyzed by reversed-phase HPLC on a Millennium HPLC system (Waters Corp.) with a Symmetry C₁₈ column, 3.9 × 150 mm, and a model 996 photodiode array detector. The mobile phase consisted of 20% methanol in 0.3% trifluoroacetic acid at a flow rate of 0.9 ml/min with detection at 278 nm. Quantitation was done by peak area measurements in comparison with a standard curve for EC.

Hydrolysis of Glucuronic Acid Conjugates. EC glucuronides were produced as mentioned above and were subjected to solid-phase extraction, dried under N₂ gas, and redissolved in 1.5 ml of 1 M sodium acetate buffer (pH 4.5). Aliquots (0.5 ml) of each sample were incubated in the absence (control) or presence of 1000 units of β-glucuronidase for 24 h at 37°C. An aliquot of each sample was also incubated with β-glucuronidase in the presence of n-saccharic acid 1,4-lactone (1 mg). The samples were subjected to solid-phase extraction as above, reconstituted in mobile phase, and analyzed by HPLC.

Hydrolysis of Sulfate Conjugates. EC sulfation was done as mentioned above and the ethyl acetate extract dried under N₂ gas. The samples were then redissolved in 0.3 ml, pH 7.4, Tris buffer and incubated at 37°C for 30 min in the presence or absence (control) of sulfatase (0.5 μl, 0.006 U). At the end of the incubation, the reaction was stopped, and the samples were directly analyzed by HPLC. One-minute HPLC eluate fractions were collected and analyzed by liquid scintillation counting after the addition of biodegradable counting scintillant (Amersham Biosciences).

Partition Coefficient Determination. The partition coefficients for EC (50 μM) and quercetin (10 μM) were determined using 1-octanol and phosphate buffer. As quercetin was very unstable in pH 7.4 buffer, a pH 4.5 buffer was used. Equal volumes of 1-octanol (equilibrated with the buffer) and flavonoid in octanol-equilibrated buffer were shaken at room temperature for 15 min.

![Chemical structure of the tea flavonoid (−)-epicatechin (EC).](Image)
Effectively inhibited by the specific disappeared with a concomitant increase in EC. This hydrolysis was Fig. 3. The major peak 2 produced the velocity versus EC concentration curves in from these experiments.

Glucuronic Acid Conjugation by the Human. As glucuronidation had been suggested to be an important pathway in the metabolism of EC in humans (Lee et al., 1995; Yang et al., 1998; Baba et al., 2000; Chow et al., 2001) but never studied at the biochemical level; we examined glucuronidation in pooled human liver microsomes from 11 donors. Surprisingly, there was no evidence of glucuronidation of EC over the concentration range of 50 to 1000 μM. Neither was (+)-catechin, an epimer of EC, glucuronidated. As the human intestine has two UGT isoforms, UGT1A8 and UGT1A10, not present in the liver (Tukey and Strassburg, 2001), we also examined human small intestinal as well as colon microsomes. However, there was still no evidence of glucuronidation of EC. To account for one UGT isoform not present in either the liver or the intestine, recombinant human UGT1A7 was used. There was no formation of EC glucuronides under any condition. Using identical experimental conditions, quercetin, containing five hydroxyl groups like EC and (+)-catechin, produced four isomeric glucuronides with human liver microsomes as previously reported (Galijatovic et al., 2001). Quercetin also formed multiple glucuronides with the human small intestinal microsomes as well as with recombinant UGT1A7.

Very low lipid solubility may be a factor limiting the access of EC to the UGT enzymes. However, the partition coefficient between octanol and pH 4.5 buffer for EC (1.1) was not much different from quercetin (2.6). The use of alamethicin to improve access of substrates to the UGT enzymes (Fisher et al., 2000) had no effect on EC glucuronidation.

Glucuronic Acid Conjugation by the Rat. The observation that no glucuronidation of EC by any of the human enzyme sources occurred was in sharp contrast to the well documented formation of a glucuronic acid conjugate when EC was given in vivo to the rat (Harada et al., 1999; Okushio et al., 1999). We therefore examined the conjugative metabolism of EC by the rat using the same experimental conditions as for the human studies. The glucuronidation of EC with pooled rat liver microsomes was linear with time for up to 60 min. In the presence of UDPGA, there were two metabolites formed, peaks 1 and 2 in Fig. 2B, which were not present in the absence of UDPGA, Fig. 2A. When EC was incubated with UDPGA and liver microsomes from Aroclor 1254-induced rats, the major peak 2 increased dramatically, and an additional small peak 3 appeared, Fig. 2C. When samples were incubated with beef liver β-glucuronidase, peak 2 disappeared with a concomitant increase in EC. This hydrolysis was effectively inhibited by the specific β-glucuronidase inhibitor d-saccharic acid 1,4-lactone, confirming peak 2 as a glucuronic acid conjugate. Peaks 1 and 3 were too small to draw any clear conclusions from these experiments.

Apparent enzyme kinetic studies focusing on the formation of the major peak 2 produced the velocity versus EC concentration curves in Fig. 3. The $K_m$ value for the control microsomes was 15.6 μM and the $V_{max}/K_m$ value was 26.8 μl/min/mg of protein. Interestingly, the induced microsomes gave a higher $K_m$ value (i.e., 35.8 μM), but the $V_{max}/K_m$ value still increased about 4-fold to 108 μl/min/mg of protein.

Sulfate Conjugation by the Human. The lack of glucuronidation of EC in humans put our focus next on sulfate conjugation. For this we used the ion-pair extraction method of Varin et al. (1987). The reaction with EC was linear with time for up to 30 min. EC was sulfated by the human liver cytosol, pooled from 10 donors, in the presence of [35S]PAPS as cosubstrate, as measured by liquid scintillation spectrometry of the ethyl acetate extract. When this extract was evaporated to dryness and the residue redissolved in mobile phase, we
could visualize two potential sulfate conjugates by reversed phase HPLC with specific radiometric detection, peaks I and II in Fig. 4B. These peaks were not present in the absence of EC, Fig. 4A. The relative retention times for these peaks, as compared with EC, were for peak I, 0.50, and peak II, 0.71. To confirm that peaks I and II are sulfate conjugates of EC, we treated the samples with bacterial aryl sulfatase and reanalyzed them by HPLC. The major peak II disappeared completely, and the radioactivity associated with this conjugate now all appeared, presumably as $\text{SO}_4^{2-}$ in the solvent front, Fig. 4C. Peak I was not affected by aryl sulfatase treatment. As SULT1A1 is the predominant SULT isoform in the liver (Campbell et al., 1987a), we next conducted sulfation experiments with a highly purified recombinant form of this enzyme (Lewis et al., 1996). Results identical to those produced by the liver cytosol in Fig. 4 were obtained (data not shown), i.e., demonstrating that one major sulfatase-sensitive conjugate and one minor sulfatase-insensitive conjugate of EC are formed. Similarly, we found the same peaks using the human intestinal cytosol from three donors. Interestingly, when we examined the cytosol prepared from Caco-2 cells, only the major peak II appeared. Also, when examining SULT1A3, thought to be a major SULT isoform in the human intestine (Sundaram et al., 1989), only the major peak II appeared.

The sulfation reaction with EC was characterized quantitatively with the velocity versus substrate concentration curves displayed in Fig. 5A to E, and apparent enzyme kinetic parameters are summarized in Table 1. The $K_m$ value for the liver was only 1.2 $\mu$M with that for the jejunum being much higher (i.e., 10.3 $\mu$M). The overall efficiency ($V_{\text{max}}/K_m$) was 2 times higher in the liver than in the intestine. The Caco-2 cells had a $K_m$ value even higher than the jejunum, 33.5 $\mu$M. The reason for the low $V_{\text{max}}/K_m$ value of the model Caco-2 cells of only 0.87 is worth noticing. The $K_m$ value for recombinant SULT 1A1 of 1.8 $\mu$M was very similar to that of the liver cytosol, whereas the $K_m$ value for recombinant SULT1A3 of 60 $\mu$M was higher than for any of the tissue preparations. The $V_{\text{max}}$ and $V_{\text{max}}/K_m$ values were high for these recombinant enzymes, reflecting their high expression in these preparations, in particular for SULT1A1 (Lewis et al., 1996).

The interindividual variation in sulfation of EC by the three jejunal cytosols examined was very large as compared with the other preparations, with $K_m$ values of 26.6, 1.5, and 3.0 $\mu$M (mean 10.3 $\mu$M, see Table 1).

Sulfate Conjugation by the Rat. To determine the importance of sulfate conjugation in the rat, we prepared rat liver cytosols from three
The first aim of our research focused on glucuronidation, which has been shown to be a major metabolic pathway for flavonoids such as chrysia (Galijatovic et al., 1999), galangin (Otake et al., 2002), and quercetin (Galijatovic et al., 2001). To our surprise, EC was not glucuronidated by either of these preparations. The Km value for sulfation of EC by these preparations was 8.8 ± 0.6 μM (i.e., higher than for the human liver cytosol (cf. Table 1]). The Vmax value was 47 ± 6 pmol/min/mg of protein and the Vmax/Km value was 5.3 μl/min/mg of protein. When these preparations were subjected to HPLC as described for the human tissues above, the chromatograms in Fig. 6 were obtained. The main peak I had a retention time identical to the minor peak I in the human tissues (cf. Figure 4). A broad peak appeared at a similar retention time as peak II in the human. When these samples were treated with aryl sulfatase, the broad peak II disappeared completely with the appearance of radioactivity in the solvent front, just as with the human tissues. The major peak I in the rat, like the same peak in the human samples, was resistant to enzymatic hydrolysis.

**Discussion**

It is essential to know the bioavailability of the flavonoids to understand their potential actions in vivo. Although EC has been detected in plasma and urine in humans after ingestion of decaffeinated green tea (Lee et al., 1995; Nakagawa et al., 1997; Yang et al., 1998; Baba et al., 2000; Chow et al., 2001), the oral bioavailability is low (Warden et al., 2001). In our previous studies using Caco-2 cells, a well accepted model of human intestinal absorption (Artursson, 1990; Artursson and Karlsson, 1991; Lennernas, 1997; Yee, 1997), we demonstrated that EC has very limited apical to basolateral transport; EC appeared to be a substrate of the apical transcellular transport; EC appeared to be a substrate of the apical transporter MR2 (Vaidyanathan and Walle, 2001). The low absorption even in the presence of MR2 inhibition suggested that the oral bioavailability of EC may be limited not only by poor transport but also by its metabolism. To explore the metabolism of EC, we focused our attention on glucuronic acid and sulfate conjugation of EC, the pathways suggested to be of greatest importance, using human liver and intestinal microsomes and cytosols as well as recombinant UGT and SULT enzymes.

The first aim of our research focused on glucuronidation, which has been shown to be a major metabolic pathway for flavonoids such as chrysia (Galijatovic et al., 1999), galangin (Otake et al., 2002), and quercetin (Galijatovic et al., 2001). To our surprise, EC was not glucuronidated by human liver microsomes, which express multiple UGT1A isoforms (Tukey and Strassburg, 2001) even when examining a large concentration range (50–1000 μM). Quercetin, a structurally related flavonoid with five hydroxyl groups like EC was as previously shown (Galijatovic et al., 2001) efficiently conjugated with the formation of four isomeric glucuronides. The experiment with quercetin also showed that the microsomes used had high catalytic activity. Certain UGT1A isoforms (i.e., UGT1A8 and UGT1A10) are not expressed in the liver but in the small intestine and colon (Tukey and Strassburg, 2001). EC was not glucuronidated by either of these tissues, nor by UGT1A7 expressed specifically in the stomach (Tukey and Strassburg, 2001).

**TABLE 1**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Km</th>
<th>Vmax</th>
<th>Vmax/Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human liver cytosol</td>
<td>1.20 ± 0.17</td>
<td>29 ± 0.2</td>
<td>23.4</td>
</tr>
<tr>
<td>Human jejunal cytosol</td>
<td>10.30 ± 8.2</td>
<td>140 ± 69</td>
<td>13.6</td>
</tr>
<tr>
<td>Caco-2 cytosol</td>
<td>33.50 ± 1.4</td>
<td>29 ± 0.7</td>
<td>0.87</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>1.80 ± 0.1</td>
<td>10501 ± 580</td>
<td>5834.0</td>
</tr>
<tr>
<td>SULT1A3</td>
<td>60.0 ± 3</td>
<td>3306 ± 66</td>
<td>55</td>
</tr>
</tbody>
</table>

EC sulfation by human liver, jejunal and Caco-2 cytosols and recombinant SULT isoforms

Apparent enzyme kinetic parameters were obtained by fitting the data, as in Fig. 5, to the Michaelis-Menten equation. Shown as mean ± S.E.M. (N = 3).

**Fig. 6.** Reversed phase HPLC tracings of products derived from incubations of rat liver cytosol with 1 μM [35S]PAPS in (A) the absence of EC, (B) the presence of EC (5 μM), and (C) after treating sample (B) with aryl sulfatase.

Detection was by fraction collection and liquid scintillation spectrometry. Retention time of unmetabolized EC was 16.5 min.
(Fisher et al., 2000) suggests lack of binding of EC to the active site of the enzyme due to some structural incompatibility as the reason for no activity.

The lack of glucuronidation of EC in humans led us to determine whether glucuronidation occurred in the rat. The rat UGT enzyme family, like human UGTs, is classified into subfamilies 1 and 2 and is responsible for glucuronidation of a wide range of compounds (Mackenzie et al., 1997). Consistent with previous observations in the intact rat (Chen et al., 1997; Zhu et al., 2000), EC was glucuronidated by rat liver microsomes. The \( K_m \) value of 15.6 \( \mu M \) was rather low, and the \( V_{\text{max}} / K_m \) value of 26.8 \( \mu M/min/mg \) of protein, if compared with glucuronidation of a variety of substrates by human liver microsomes (Soars et al., 2001), would classify this glucuronidation rate as efficient. Liver microsomes from Aroclor 1254-induced rats increased the glucuronidation of a variety of substrates by human liver microsomes (Soars et al., 2001), would classify this glucuronidation rate as efficient. Liver microsomes from Aroclor 1254-induced rats increased this efficiency to a very high value of 108 \( \mu M/min/mg \) of protein.

The lack of glucuronidation of EC by all human tissue preparations and UGT isoforms led us to focus on sulfation conjugation. EC was efficiently sulfated by the human liver as well as the intestinal cytosol, similar to previous data in Caco-2 cells (Vaidyanathan and Walle, 2001), however, with quite different apparent \( K_m \) values (i.e., 1.2 versus 10.3 \( \mu M \)) indicating the involvement of different SULT isoforms. It has long been known that the main SULT isoform in the human liver is SULT1A1 (P-form phenol sulftransferase) (Campbell et al., 1987a). Consistent with this, our observations showed that the \( K_m \) values for the liver and recombinant SULT1A1 were virtually identical. The higher \( K_m \) value for the intestine indicates the participation of an additional SULT isoform. It is indeed well known that the intestine has a high expression of SULT1A3 (human monoamine-form phenol sulftransferase) (Sundaram et al., 1989), which had a rather high \( K_m \) value of 60 \( \mu M \) for the sulfation of EC. Both ion-exchange chromatography (Sundaram et al., 1989) and Western analysis (Ganguly et al., 1995) clearly demonstrate that both SULT1A1 and SULT1A3 have high expressions in the human small intestine. Our observations of \( K_m \) values of 1.5, 3.0 and 26.6 \( \mu M \) in the cytosols from three subjects therefore may suggest polymorphic distribution of these SULT isoforms, a phenomenon clearly demonstrated for SULT1A1 (Ozawa et al., 1998; Raftogianis et al., 1999), although not for the intestine. This deserves further investigations. Other known SULT isoforms such as SULT2A1 and 1E1, did not contribute significantly. One SULT isoform, the thyroid hormone sulftransferase (Wang et al., 1998) was not examined. This isoform may have contributed some to the activities observed.

Although the human liver is more efficient \( (V_{\text{max}} / K_m) \) in removing low concentrations of EC than the intestine (Table 1), the intestine is more efficient for higher concentrations, with a \( V_{\text{max}} \) value exceeding that of the liver by about 5-fold. Concentrations in the intestinal lumen of EC from the diet most likely will exceed the \( K_m \) value for sulfation. Thus, this presystemic site should be a critically important determinant of the exposure of the intestine, including the colon, to EC, whereas the liver effectively will further reduce the levels of EC appearing in the systemic circulation.

The sulfation method that we used was an ion-pair extraction method (Varin et al., 1987), which we find superior to the much more commonly used barium precipitation method (Foldes and Meek, 1973). Attempts to extend these methods to HPLC failed with the barium precipitation method, possibly due to hydrolysis of the sulfate conjugates formed. However, this worked quite well with the ion-pair extraction method, as shown for another flavonoid in a preliminary communication (Otakе et al., 2002). This extension to HPLC increases the molecular specificity of the method greatly. It is thus interesting to note that two sulfate conjugates are produced by the liver, intestine, and recombinant SULT1A1, whereas only one of them is produced by the Caco-2 cells and SULT1A3. Although the mRNA for SULT1A1 has been found in the Caco-2 cells (Satoh et al., 2000), there is no evidence of the protein expression in these cells. We find very low expression of SULT1A1 in these cells (unpublished results).

The reason for the resistance of the minor sulfate conjugate peak to cleavage by aryl sulfatase is not clear. Attempts to identify these conjugates by HPLC/mass spectrometry have failed as sulfate conjugates of flavonoids are labile during mass spectrometry analysis (Galijatovic et al., 1999). Interestingly, sulfate conjugates of isoflavonoids are quite stable under HPLC/mass spectrometry conditions (Coward et al., 1996).

The Caco-2 cells sulfated EC with a product identical to the one previously shown to be formed under transport conditions (Vaidyanathan and Walle, 2001). However a very low \( V_{\text{max}} \) value suggests that the Caco-2 cells have a low expression of SULT isoforms. This may limit the utility of this cell line as a useful model of the human intestine and should be further examined.

Finally, sulfation conjugation of EC also occurred in the rat liver. The apparent \( K_m \) value for this reaction was 8 times higher than in the human liver. This is similar to a previous investigation comparing the sulfation of a variety of compounds between the humans and the rat (Campbell et al., 1987b). However, the sulfate conjugates formed in the rats appear to be different from those found in the humans. The major peak formed is resistant to enzymatic hydrolysis as compared with the minor one in the humans. The broad peak formed in the rat corresponds to the retention time of the major peak in the humans. One reason for such a broad peak could be the presence of more than one isomer. These data taken together would support the idea that glucuronidation is more important than sulfation of EC in the rat, which is opposite to the situation in the humans.

In summary, this study adds significantly to our understanding of human presystemic metabolism of EC and possibly tea flavonoids in general. These results indicate that sulfation and not glucuronidation is the major metabolic pathway for EC in humans. It will be important to extend these studies to the other tea flavonoids. Also, whereas glucuronidation of EC occurred in the rat, it did not in humans. Further work is being done in our laboratory with respect to understanding the flavonoid substrate specificity for human UDP-glucuronosyltransferases.

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References


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References


