Variable Sulfation of Dietary Polyphenols by Recombinant Human Sulfotransferase (SULT) 1A1 Genetic Variants and SULT1E1

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ABSTRACT:

Human cytosolic sulfotransferases (SULTs) catalyze the sulfate conjugation of several important endo- and xenobiotics. Among the superfamily of SULT enzymes, SULT1A1 catalyzes the sulfation of small planar phenolic compounds, whereas SULT1E1 has a major role in estrogen conjugation. The human SULT1A1 gene has common single nucleotide polymorphisms that define three allozymes, SULT1A1*1, *2, and *3. The enzyme kinetics of SULT1A1 allozymes and SULT1E1 were characterized for the polyphenolic substrates apigenin, chrysin, epicatechin, quercetin, and resveratrol. Purified recombinant SULT proteins were generated in a baculoviral-insect cell system, and incubated in vitro with each substrate to determine catalytic activity. The effect of polyphenol sulfation was examined in mammalian cell lines stably expressing SULT1E1. For all polyphenols investigated, “normal-activity” SULT1A1*1 allozyme had significantly greater V_max estimates than SULT1E1, and allele-specific differences in SULT1A1-mediated sulfation were observed. The polymorphic SULT1A1*2 allozyme exhibited low activity toward apigenin, epicatechin, and resveratrol. SULT1A1*1 and *3 acted as normal-activity allozymes for these substrates. Altered cellular proliferation was observed in MCF-7 cells stably expressing SULT1E1 upon treatment with chrysin, quercetin, or resveratrol, thus suggesting inactivation of these compounds by SULT1E1. These results suggest an important role for SULT isozymes and their pharmacogenetics in polyphenol disposition.

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ABBREVIATIONS: SULT, sulfotransferase; UGT, uridine diphosphoglucuronosyltransferase; PAPS, 3’-phosphoadenosine 5’-phosphosulfate, DMSO, dimethyl sulfoxide.
has been suggested that dietary polyphenols alter the bioavailability of endogenous estrogens by competing as substrates and/or inhibitors for human SULT1E1 and SULT1A1 isozymes, thus possibly contributing to variable breast cancer risk (Harris et al., 2004). Hepatic and extrahepatic conjugation of polyphenols such as resveratrol is thought to contribute to their low oral bioavailability (Walle et al., 2004). Resveratrol has recently received much attention for its chemopreventive and numerous other health benefits despite its low bioavailability (Baur et al., 2006; Baur and Sinclair, 2006).

Several studies have identified the potential of dietary polyphenols as chemopreventives and the importance of their conjugation in decreased bioavailability. However, it remains to be determined how phase II metabolizing enzyme pharmacogenetics alters polyphenol disposition. We have previously characterized the in vitro enzyme kinetics of SULT1A1-mediated sulfation for the flavonoids chrysin, genistein, and quercetin (Nagar et al., 2006). In the previous study, we further characterized the SULT1A1*1, *2 or *3 allozyme-dependent variation in sulfation of these flavonoids. The purpose of this study is to extend our understanding of polyphenol sulfation and the cellular effect of this process. We hypothesize that both SULT1A1 and SULT1E1 play important roles in the conjugation of numerous dietary polyphenols. We additionally hypothesize that SULT1A1 pharmacogenetics explains variable sulfation of these polyphenols. We therefore characterized in detail the enzyme kinetics of SULT1A1 (*1, *2, and *3 allozymes)- and SULT1E1-mediated sulfation of the following flavonoids: flavones (apigenin and chrysin) and flavonols (epicatechin and quercetin). Additionally, the sulfation of the nonflavonoid polyphenol resveratrol was evaluated.

Materials and Methods

Chemicals and Reagents. Apigenin, chrysin, quercetin dihydrate, (−)-epicatechin, and resveratrol were obtained from Sigma-Aldrich (St. Louis, MO). The cosubstrate 3′-phosphoadenosine 5′-phosphosulfate (PAPS) had previously been purchased from Dr. H. Glatt (German Institute of Human Nutrition, Nuthetal, Germany). Radiolabeled [35S]PAPS was obtained from PerkinElmer Life And Analytical Sciences (Boston, MA). Analytical grade chemicals were used to prepare buffers and other reagents. SF-9 cells and related media were obtained from Invitrogen (Carlsbad, CA). MCF-7 cells were obtained from American Type Culture Collection (Manassas, VA), and its medium was obtained from Mediatech (Herndon, VA). Gibco RPMI 1640 for cell proliferation assays (with L-glutamine, without phenol red) was obtained from Invitrogen. All other cell culture reagents were purchased through Fisher Scientific International, Inc. (Hampton, NH).

Protein Purification of Recombinant SULT1A1 (*1, *2, and *3) and SULT1E1. Recombinant 6X-histidine-tagged SULT1A1*1, *2, and *3 and SULT1E1 proteins were expressed in a baculoviral-insect cell system. SULT1A1*1, *2, and *3 and SULT1E1 constructs were cloned into the baculovirus expression vector pBLUEBac-His2A and cotransfected with 1 of BacVector-3000 viral DNA through liposome-mediated transfection into SF-9 insect cells as described previously (Raftogianis et al., 1999; Nagar et al., 2006). Stocks of viral 6X-His-tagged SULT cDNA were used to infect SF-9 cell cultures at 28°C for 48 h and were harvested for protein purification. Bradford assays were performed on the purified proteins to determine their concentrations. Further analysis to verify the purity of the proteins included SDS-polyacrylamide gel electrophoresis and Western blot with anti-SULT1A1 or anti-SULT1E1 antibodies. The purified proteins resulted in a single SULT1A1 or SULT1E1 band for each sample in Western blot analyses. Aliquots of purified protein were stored at −80°C for future use.

Radiometric SULT Assays. The purified recombinant SULT proteins were characterized for catalytic activity toward the flavonoids and resveratrol with a radiometric assay (Anderson and Weinshilboum, 1980; Varin et al., 1987; Geese and Raftogianis, 2001). Initially, the conditions for linearity of protein amount and time were optimized. The reaction components were purified protein, substrate (apigenin, chrysin, quercetin dihydrate, (−)-epicatechin, or resveratrol) and cosubstrate [35S]PAPS contained in 0.2-ml thin-wall polymers.

Fig. 1. Kinetics of sulfation of dietary flavone apigenin by SULT1E1 and SULT1A1 isozymes. Purified recombinant SULT1A1*1, *3, SULT1E1 (0.5 μg each), or SULT1A1*2 (1 μg) protein was incubated with substrate and 10 μM [35S]PAPS for 15 min at 37°C, and the formation of radioactive sulfated product was quantitated with a radiometric assay as described under Materials and Methods. A, Michaelis-Menten curves for apigenin sulfation by SULT1E1, SULT1A1*1, *2, or *3 at 0 to 50 μM. Inset, Michaelis-Menten curve for the *2 allozyme is depicted on an expanded y-axis scale at 0 to 100 μM apigenin. Data are expressed as means ± S.D.; n = 3 for all except n = 6 for *2. Representative fitted lines are depicted; parameter estimates were obtained by fitting the kinetic models to actual data replicates. B, Eadie-Hofstee plots of Michaelis-Menten kinetics of apigenin sulfation by SULT1E1 or SULT1A1*1, *2, or *3. Data reported as means; n = 3 for all except n = 6 for *2. C, apigenin sulfation by SULT1E1, SULT1A1*1, or *3, at 0 to 100 μM substrate concentration, where inhibition is shown. Data are reported as means; n = 3 for all except n = 6 for *3. A substrate inhibition equation (see Materials and Methods) could not adequately describe these data; therefore, K_i values were not obtained.
The final concentration of DMSO was 3.3% in all incubations. The range of total protein tested was 0.2 to 2 μg. The reactions were initiated by the addition of cosubstrate 10 μM PAPS (1512531 [PAPS] + cold PAPS in the ratio 1:9 v/v). Preliminary experiments were conducted with 10 to 30 μM PAPS; 10 μM PAPS was found to be sufficiently high and was not depleted at the highest substrate concentrations studied. The reactions were quenched with the addition of 3 μl of 2.5% aqueous glacial acetic acid, 6 μl of fresh 0.1 M tetrahydroammonium phosphate monobasic, and 150 μl of ethyl acetate. Vortexing and centrifugation at 1800g for 3 min of the tubes resulted in separate aqueous and organic layers. To quantitate the reaction products, 100 μl of the top organic layer was added to scintillation fluid for liquid scintillation counting. All incubations were carried out in triplicate, with appropriate negative controls (no substrate).

**Data Analysis for Enzyme Kinetics.** Initially, a broad range of substrate concentrations was used to determine inhibitory substrate concentration. When inhibition was observed, the substrate inhibition model was used to try to obtain Ki estimates (Nagar et al., 2006). If the substrate inhibition equation failed to fit the observed data, the Michaelis-Menten equation was fit to a narrow range of substrate concentrations; parameter estimates were obtained by the following equation used to fit the data: v = Vmax · [S]/(Km + [S]), where v is the rate of the reaction, Vmax is the maximum velocity estimate, [S] is the substrate concentration, and Km is the Michaelis-Menten constant. Before nonlinear regression analysis, all data were transformed and Eadie-Hofstee curves plotted; the Michaelis-Menten model was fit only to data exhibiting linear Eadie-Hofstee plots. Nonlinear regression was performed with GraphPad Prism for Windows (version 4.03; GraphPad Software Inc., San Diego, CA). A statistical comparison of the parameter estimates was performed using a two-sided t test assuming normal distribution, for which a p value < 0.01 was deemed significant (Nagar et al., 2006).

**Proliferative Response of MCF-7 Cells Stably Expressing SULT1E1 to Polyphenols.** Generation of MCF-7 cells stably expressing SULT1A1*1, *2, or *3 has been described previously (Nagar et al., 2006). MCF-7 cells stably expressing SULT1E1 were similarly generated. Multiple clones were isolated and expanded. Clones were characterized with Southern, Northern, and Western blot analyses, and three SULT1E1 clones (nos. 1, 10, and 22) were found to express RNA comparable to the cell lines expressing SULT1A1. Thus, these three clones were evaluated in subsequent studies. MCF-7 cells stably expressing SULT1E1 were treated with polyphenols (0–100 μM) for 48 h, and cell viability was determined with a standard Alamar Blue (BioSource International, Camarillo, CA) assay. MCF-7 cells as negative controls and MCF-7 cells stably expressing SULT1E1 (clones 1, 10, and 22) and empty vector pCR3.1 were plated in 96-well plates. Cells were grown in RPMI 1640 (containing l-glutamine, but without phenol red) medium with 5% charcoal-dextran-stripped fetal bovine serum overnight at 5% CO2 and 37°C. The cell culture medium was replaced every 12 h to remove endogenous estrogens. The next day, the cells were dosed with resveratrol, apigenin, chrysirin, epicatechin, or quercetin (0–100 μM) prepared in fresh media. After 48 h, fluorescence was measured at an excitation wavelength of 530 nm and emission at 590 nm. Proliferation data were normalized such that cell growth with no treatment was set at 100% for each cell line at each substrate concentration. All experiments were carried out in four replicates per assay condition. Proliferation among cell lines was compared statistically by analysis of variance ANOVA followed by Tukey’s multiple comparison test (GraphPad Prism for Windows, version 4.03).

**Results**

Sulfation kinetics of three polyphenols—apigenin, epicatechin, and resveratrol—via recombinant SULT1A1 and SULT1E1 were initially determined. Figure 1A depicts the sulfation of apigenin by SULT1E1 and SULT1A1*1, *2, and *3, with the respective Eadie-Hofstee plots depicted in Fig. 1B. Michaelis-Menten estimates are reported in Table 1. The Vmax estimate for apigenin sulfation was significantly lower with SULT1E1 and SULT1A1*2 compared with that for the *1 protein. The Km estimate with SULT1E1 was significantly higher than that with SULT1A1*1. Inhibition was observed at high apigenin concentrations with all the proteins (Fig. 1C); inhibition could not be determined with SULT1A1*2 because of low activity. A substrate inhibition model could not explain these inhibition data; therefore, no Ki estimates were obtained.

Figure 2 depicts the kinetics of epicatechin sulfation. In Fig. 2A, epicatechin sulfation by SULT1E1 as well as by SULT1A1*1, *2, and *3 has not exhibited inhibition in the concentration range studied. Eadie-Hofstee plots are shown in Fig. 2B. Comparison of the kinetic estimates (Table 1) revealed significantly lower Vmax estimates and significantly higher Km estimates for SULT1E1 and SULT1A1*2 compared with those for the SULT1A1*1 allozyme. Interestingly, the Km estimate for the SULT1A1*1*3 allozyme was significantly lower than that for SULT1A1*1, leading to a Vmax/Km estimate comparable with that of the wild-type *1 allozyme.

Resveratrol sulfation kinetics is depicted in Fig. 3, with corresponding kinetic estimates reported in Table 1. As can be seen in Fig. 3A, resveratrol sulfation by either SULT1E1 or the SULT1A1 allozymes did not exhibit inhibition at the substrate concentrations evaluated. Figure 3B shows the Eadie-Hofstee plots for these data. The kinetic estimates for resveratrol sulfation via SULT1E1 were significantly

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**TABLE 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Estimate</th>
<th>Sulfotransferase</th>
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<tbody>
<tr>
<td></td>
<td>SULT1E1</td>
<td>SULT1A1*1</td>
</tr>
<tr>
<td>Apigenin</td>
<td></td>
<td></td>
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<tr>
<td>Vmax</td>
<td>5.83 ± 0.27</td>
<td>0.049 ± 0.002b</td>
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<tr>
<td>Km</td>
<td>3.72 ± 0.17a</td>
<td>2.64 ± 0.59</td>
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<tr>
<td>Epicatechin</td>
<td>0.36 ± 0.024a</td>
<td>0.052 ± 0.002a</td>
</tr>
<tr>
<td>Resveratrol</td>
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<td>5.96E-05</td>
</tr>
<tr>
<td>Chrysin</td>
<td>1.6 ± 0.10a</td>
<td>8.4 ± 0.14a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3.2 ± 0.15a</td>
<td>3.5 ± 0.8a</td>
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*a Estimate significantly different from *1 as determined by a two-sided t test: p < 0.01.
*b Data are expressed as estimate ± S.E.; n = 6.
*c Data reported by Nagar et al. (2006).
SULTATION OF DIETARY POLYPHENOLS BY SULT1A1 AND SULT1E1

Fig. 2. Kinetics of sulfation of dietary flavonol epicatechin by SULT1E1 and SULT1A1 allozymes. Purified recombinant SULT1A1*1, *3 (0.5 μg each), SULT1A1*2, or SULT1E1 (2 μg each) protein was incubated with substrate and 10 μM [35S]PAPS for 15 min at 37°C, and the formation of radioactive sulfated product was quantitated with a radiometric assay as described under Materials and Methods. A, Michaelis-Menten curves for epicatechin sulfation by SULT1E1 or SULT1A1*1, *2, or *3 at 0 to 3 mM. Insets, Michaelis-Menten curves for the *2 allozyme and SULT1E1 are depicted on an expanded y-axis scale. Data are expressed as means ± S.D., n = 3. Representative fitted lines are depicted; parameter estimates were obtained by fitting the kinetic models to actual data replicates. B, Eadie-Hofstee plots of Michaelis-Menten kinetics of resveratrol sulfation by SULT1E1 or SULT1A1*1, *2, or *3. Data are reported as means; n = 3.

The effects of all five polyphenols on cell proliferation were evaluated next. MCF-7 cells exhibited similar proliferation profiles as cells expressing the empty vector pCR3.1 (data not shown). As shown in Fig. 5A, all five polyphenols inhibited cell proliferation at the highest concentration evaluated (100 μM). At 50 μM, quercetin induced proliferation, whereas resveratrol inhibited cell proliferation at 50 μM concentration. Figure 5B shows the relative proliferation of negative control pCR3.1 empty vector-expressing MCF-7 cells versus cells stably expressing SULT1E1. Three SULT1E1 clones (nos. 1, 10, and 22) were evaluated. There was no difference in proliferation among the cell lines upon treatment with 50 μM concentrations of either apigenin or epicatechin. Quercetin (50 μM) induced proliferation in the negative control pCR3.1-expressing cells. This proliferation was inhibited by SULT1E1-expressing cells (clones 10 and 22) upon similar treatment. Chrysin and resveratrol both inhibited proliferation of pCR3.1-expressing cells, whereas all clones expressing SULT1E1 proliferated significantly more than the control cell line upon treatment.
Dietary polyphenols have antioxidant properties and have been studied for their anticancer potential (Beecher, 2003; Baur and Sinclair, 2006). These compounds are sulfated by human SULT1A1 and SULT1E1 and are thought to interact with estrogens via this common metabolic pathway (Otake et al., 2000; Harris et al., 2004). SULT1A1 pharmacogenetics have been evaluated in detail (Raftogianis et al., 1996, 1997, 1999). Recently, genetic mutations in the human SULT1E1 gene, albeit at low frequencies, have been reported (Adjei et al., 2003). Although epidemiological studies have examined the association between SULT1A1 pharmacogenetics, dietary intake, and cancer risk, few studies have examined the genotype-phenotype correlation between polymorphic SULT1A1 and dietary polyphenol metabolism (Seth et al., 2000; Bamber et al., 2001; Williams et al., 2001; Zheng et al., 2001; Wang et al., 2002; Tang et al., 2003; Wu et al., 2003; Choi et al., 2005; Nowell et al., 2005; Shatalova et al., 2005). We have previously reported allele-specific differences in SULT1A1-catalyzed sulfation of chrysin and quercetin (Nagar et al., 2006). In the current study, we have characterized the sulfation of additional flavonoids by purified recombinant SULT1A1 and SULT1E1 proteins.

With the purified recombinant proteins expressed in a baculoviral-Sf-9 insect cell system, the "normal-activity" SULT1A1*1 allozyme exhibited significantly greater $V_{\text{max}}$ estimates for all the polyphenols studied compared with the SULT1E1 protein (Table 1). For epicatechin, resveratrol, and chrysin sulfation by SULT1E1, data are reported as means; $n = 3$. C, sulfation of quercetin and chrysin by SULT1E1 at 0 to 100 $\mu$M substrate concentration, where inhibition is shown. Data are reported as means; $n = 6$. A substrate inhibition equation (see Materials and Methods) could not adequately describe these data; therefore, $K_i$ values were not obtained.

Discussion

Dietary polyphenols have antioxidant properties and have been studied for their anticancer potential (Beecher, 2003; Baur and Sinclair, 2006). These compounds are sulfated by human SULT1A1 and SULT1E1 and are thought to interact with estrogens via this common metabolic pathway (Otake et al., 2000; Harris et al., 2004). SULT1A1 pharmacogenetics have been evaluated in detail (Raftogianis et al., 1996, 1997, 1999). Recently, genetic mutations in the human SULT1E1 gene, albeit at low frequencies, have been reported (Adjei et al., 2003). Although epidemiological studies have examined the association between SULT1A1 pharmacogenetics, dietary intake, and cancer risk, few studies have examined the genotype-phenotype correlation between polymorphic SULT1A1 and dietary polyphenol metabolism (Seth et al., 2000; Bamber et al., 2001; Williams et al., 2001; Zheng et al., 2001; Wang et al., 2002; Tang et al., 2003; Wu et al., 2003; Choi et al., 2005; Nowell et al., 2005; Shatalova et al., 2005). We have previously reported allele-specific differences in SULT1A1-catalyzed sulfation of chrysin and quercetin (Nagar et al., 2006). In the current study, we have characterized the sulfation of additional flavonoids by purified recombinant SULT1A1 and SULT1E1 proteins. Importantly, the effect of SULT1E1-catalyzed sulfation (and inactivation) of polyphenols on human breast cancer cells is additionally reported.

With the purified recombinant proteins expressed in a baculoviral-SI-9 insect cell system, the "normal-activity" SULT1A1*1 allozyme exhibited significantly greater $V_{\text{max}}$ estimates for all the polyphenols studied compared with the SULT1E1 protein (Table 1). For epicatechin, resveratrol, and chrysin, the $V_{\text{max}}/K_m$ estimates were at least 1 order of magnitude greater with SULT1E1*1 than with SULT1E1. Specific activities for epicatechin and quercetin have been reported with purified recombinant SULTs in transformed BL21 (DE3) cells (Pai et al., 2001). At 100 $\mu$M epicatechin, our mean specific activity value for sulfation by SULT1E1 (300 pmol/min/mg protein) compares very well with that published by Pai et al. (351 pmol/min/mg protein). Quercetin sulfation by recombinant SULT1E1 at 100 $\mu$M substrate yielded a specific activity of 90 pmol/min/mg in our assays, compared with 610 pmol/min/mg reported by Pai et al. Differences in incubation conditions may account for this difference. To our knowledge, this study is the first report of apigenin sulfation kinetics catalyzed by
residues. Chrysin sulfation has been reported in human liver samples as well as in human liver cell lines, and the $V_{\text{max}}$ estimates we report are higher than those reported in human livers, possibly because we used purified recombinant protein in our assays (Galiatov et al., 1999; De Santi et al., 2000).

With regard to SULT1A1-catalyzed polyphenol sulfation, to our knowledge allelic-specific sulfation of apigenin, epicathechin, and resveratrol by SULT1A1*1, *2, and *3 alleles has not been reported to date. Our results indicate a significantly lower $V_{\text{max}}$ estimate for apigenin sulfation by SULT1A1*2 compared with the *1 allozyme. With epicatechin as the substrate, SULT1A1*2 exhibited a lower $V_{\text{max}}$ and a higher $K_m$ than the *1 variant, leading to a very low $V_{\text{max}}/K_m$ ratio. SULT1A1*3, on the other hand, had a significantly lower $K_m$ than *1, and a comparable $V_{\text{max}}/K_m$ estimate. Mean SULT1A1*1 activity toward 100 $\mu$M epicatechin (1173 pmol/min/mg) compared well with a previously reported value (1364 pmol/min/mg) (Pai et al., 2001). Resveratrol sulfation by SULT1A1*1 and *3 alleles showed comparable intrinsic clearance ($V_{\text{max}}/K_m$) estimates despite significantly different $V_{\text{max}}$ estimates. The SULT1A1*2 allozyme had a significantly lower $V_{\text{max}}$ for resveratrol sulfation compared with *1. Thus, allele-specific differences in SULT1A1-mediated activity were observed for all the polyphenols studied. Data presented here are in agreement with literature reports in which SULT1A1*2 has been shown to be the “low-activity” allele toward different classes of substrates (Rafiqogianis et al., 1999).

Conjugation of the polyphenol resveratrol at different positions, leading to various metabolites, has recently been described (Miksits et al., 2005). Miksits et al. reported the formation of two sulfated metabolites via SULT1A1, with substrate inhibition and sigmoidal kinetics. That study generated recombinant SULT1A1 protein in a bacterial system, whereas we report the use of a baculoviral expression system. The variable protein source in the two studies partly explains the different results. It is also important to consider possible solvent effects: we used DMSO at a relatively high final concentration (3.3%) in our incubations. Miksits et al. used a high-performance assay for metabolite detection, whereas the radiometric assay used here does not allow for differentiation between different sulfated metabolites. Despite this drawback, the estimates obtained here were useful in a comparative study among the SULT1E1 and SULT1A1*1, *2, and *3 allozymes. At the substrate concentration range evaluated, data transformation yielded linear Eadie-Hofstee plots (Figs. 1B, 2B, 3B, and 4B), and the Michaelis-Menten equation fit the data well. Kinetic estimates obtained here and those reported with recombinant SULT proteins in other studies, yielded comparable results (Pai et al., 2001). Additionally, as discussed below, cellular phenotypic data in cultured mammalian cells for substrates such as quercetin and resveratrol correlate well with data from recombinant protein experiments. Current studies in our laboratory are focused on developing high-performance liquid chromatography-based assays to quantitate polyphenol conjugates for future work.

Although data obtained from purified recombinant protein provide useful enzyme kinetic parameters, it is critical to examine the effect of enzymatic inactivation of a substrate in vivo. Cultured cells provide an environment closer to the in vivo situation compared with purified protein. Additionally, in the case of potentially chemopreventive polyphenols, it is possible to evaluate the cellular effect of the substrate (in this case the proliferative response) in cells in the presence or absence of the enzyme. To this end, we initially characterized the effect of varying concentrations of polyphenols on MCF-7 cells stably expressing pcR3.1 (Fig. 5A). Resveratrol has been reported to have an antiproliferative effect on MCF-7 cells (Kim et al., 2004). Similar effects were observed in our assays in both MCF-7 cells and cells stably expressing pcR3.1. Next, the effect of polyphenol sulfation (and inactivation) was evaluated. At 50 $\mu$M concentration, chrysin, apigenin, and resveratrol had an antiproliferative effect on control cells, whereas quercetin induced proliferation. Cells stably expressing SULT1E1 reversed the effects of chrysin, quercetin, and resveratrol (Fig. 5B). Thus, sulfation of these polyphenols by SULT1E1 yielded a phenotypic response at the cellular level, and inactivation by SULT1A1-catalyzed sulfation reversed the proliferative effect. SULT1E1 is the major isozyme responsible for the sulfation of endogenous estrogens (Coughtrie, 2002). Therefore, it would be interesting to investigate the effect of estrogen plus polyphenol cotreatment in cells stably expressing SULT1E1 to determine what effect this interaction elicits at the cellular level. It is possible that other mechanisms besides sulfation play a major role in the disposition and hence the effect of these polyphenols in MCF-7 cells. MCF-7 cells exhibit low glucuronidation capacity and UGT2B15 expression, and flavonoids are substrates for this human UGT isozyme (Harrington et al., 2006). Additionally, flavonoids such as chrysin (and its conjugated metabolites) are good substrates for the multidrug resistance protein MRP2 transporter (Walle et al., 1999), and chrysin is known to inhibit the breast cancer resistance protein BCRP (ABCG2) in MCF-7 cells (Zhang et al., 2004).

In conclusion, in this study we evaluated the sulfation of five polyphenols with recombinant polymorphic SULT1A1 variants and SULT1E1. For most polyphenols examined, recombinant SULT1A1 exhibited greater activity than SULT1E1. Apigenin, epicathechin, and resveratrol exhibited SULT1A1 allele-specific variation in sulfation, with SULT1A1*1 and *3 acting as normal-activity alleles and SULT1A1*2 acting as a low-activity allozyme. Altered cellular proliferation was observed in cells stably expressing SULT1E1 upon treatment with chrysin, quercetin, or resveratrol, possibly due to inactivation of these substrates by SULT1E1. Together, these results provide evidence for the role of SULT enzymes and their pharmacogenetics in polyphenol conjugation and cellular effects.

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References


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