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Sulfation of Hesperetin, Naringenin and Apigenin by the Human Cytosolic Sulfotransferases: A Comprehensive Analysis

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Abstract

Previous studies have revealed sulfation as a major pathway for the metabolism of hesperetin, naringenin and apigenin. The current study was designed to identify the human cytosolic sulfotransferase (SULT) enzyme(s) capable of sulfating these flavonoid compounds. Of the thirteen human SULTs, six (1A1, 1A2, 1A3, 1B2, 1C4, 1E1) displayed significant sulfating activity toward hesperetin, five (1A1, 1A2, 1A3, 1B2, 1C4) displayed sulfating activity towards naringenin, and four (1A1, 1A2, 1A3, 1C4) showed sulfating activity towards apigenin. Of the four human organ specimens tested, liver and intestine cytosols displayed much higher hesperetin-, naringenin- and apigenin-sulfating activity than lung and kidney cytosols. Moreover, sulfation of hesperetin, naringenin and apigenin was shown to take place in HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells under cultured conditions. Taken together, these results provided a biochemical basis underlying the metabolism of hesperetin, naringenin and apigenin through sulfation in humans.

Keywords

Hesperetin; naringenin; apigenin; sulfation; cytosolic sulfotransferase; SULT

1. Introduction

Hesperetin, naringenin and apigenin (cf. their chemical structures in Fig. S1) are members of the flavonoid family which comprises a large group of polyphenolic compounds primarily derived from plants [Harnly et al., 2006]. Hesperetin has been reported to occur naturally in citrus fruits like grapefruit, lemon, lime and orange [Justesen et al., 1998; Tomas-Barberan and Clifford, 2000]. Similarly, naringenin also can be found in citrus fruits and tomato as well. [Justesen et al., 1998; Tomas-Barberan and Clifford, 2000]. Apigenin on the other hand

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has been reported to be present in various vegetables that include celery, parsley, onion, wheat sprouts, chamomile and beverage such as tea [Hertog et al., 1993; Justesen et al., 1998; Patel et al., 2007]. Flavonoids are widely present in daily diet and have been reported to exert anti-inflammatory, anti-oxidant, anti-cancer, cardiovascular protection, neuro-protective, and antidepressant effects [Panche et al., 2016]. Previous studies have demonstrated that the metabolism of flavonoids occurs mainly through glucuronidation and sulfation [Nakazawa and Ohsawa, 2000; Xiao and Högger, 2013; Ma et al., 2016; Zeng et al., 2017]. Using authentic standards in HPLC and H-NMR analysis, hesperetin-3'-*O*- and 7'-*O*-glucuronides have been shown to be present in human and rat plasma [Brand et al., 2010]. In regard to sulfation, hesperetin has been shown to be a substrate for several human cytosolic sulfotransferase (SULT) enzymes as well as rat and human tissue samples [Brand et al., 2010; Huang et al., 2009]. For naringenin, major metabolites through glucuronidation were identified as naringenin-4'-*O*- and 7'-*O*-glucuronides as well as three naringenin-*O*-diglucuronides [Pereira-Caro et al., 2014], and naringenin sulfates have been detected in rabbit and rat plasma [Hsiu et al., 2002; Lin et al., 2014]. For apigenin, the major metabolites have been reported to be glucuronide and sulfate conjugates [Liu and Hu, 2002].

Sulfation is known to be a major Phase II metabolic pathway for the metabolism and excretion of a wide range of endogenous compounds as well as xenobiotics [Suiko et al., 2017]. The responsible SULT enzymes catalyze the transfer of a sulfonate group from the active sulfate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS), to acceptor substrate compounds containing hydroxyl or amino group [Lipmann, 1958]. Sulfate conjugation by the SULT enzymes generally leads to the inactivation of biologically active compounds and/or the increase in their water-solubility, thereby facilitating their removal from the body [Suiko et al., 2017]. In humans, 13 distinct SULTs that fall into four SULT gene families have been identified [Blanchard et al., 2004].

In this study, we investigated the sulfating activity of all thirteen known human SULTs towards hesperetin, naringenin and apigenin. The generation and release of [³⁵S]sulfated derivatives of these three flavonoids by HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells, labeled with [³⁵S]sulfate in the presence of different concentrations of hesperetin, naringenin and apigenin was examined. Furthermore, human lung, liver, kidney and small intestine cytosols were analyzed to verify the presence of hesperetin-, naringenin- and apigenin-sulfating activity.

2. Results and Discussion

Previous studies have revealed that sulfation plays an important role in the metabolism of hesperetin, naringenin and apigenin. The currently study aimed to identify the human SULTs that are capable of sulfating these flavonoid compounds. Human organ cytosols were also examined for sulfating activities towards these compounds. Moreover, sulfation of hesperetin, naringenin and apigenin under metabolic conditions was examined using cultured HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells.

2.1. Sulfating activities of the human SULTs towards hesperetin, naringenin and apigenin.

All thirteen human SULTs, expressed and purified previously [Sakakibara et al., 1998; Pai et al., 2002; Sakakibara et al., 2002; Suiko et al., 2002], were examined for sulfating activity with hesperetin, naringenin and apigenin (at a concentration of 10 μ M) as substrates. Results obtained are compiled in Table 1. Seven (1C2, 1C3, 2A1, 2B1a, 2B1b, 4A1, 6B1) of the thirteen human SULTs tested displayed no detectable sulfating activity towards any of the three compounds tested as substrates. Of the other six human SULTs (1A1, 1A2, 1A3, 1B2, 1C4, 1E1), 1A1, 1A2, 1A3 and 1C4 exhibited sulfating activity towards all three compounds. Of the other two human SULTs (1B1 and 1E1), SULT1E1 displayed sulfating activity only toward hesperetin, while SULT1B1 displayed sulfating activities toward hesperetin and naringenin. Based on these results, SULT1A1 appeared to be the major enzyme responsible for the sulfation of these three compounds, followed by SULT1A2, SULT1A3 and SULT1C4. SULT1B1 displayed low, yet significant, sulfating activity towards both hesperetin and naringenin, whereas SULT1E1 could sulfate only hesperetin. It should be pointed out that SULT1A1, SULT1A2, and SULT1A3 have previously been called the “phenol sulfotransferases” that sulfate preferentially compounds carrying aryl hydroxyl groups [Blanchard et al., 2004]. In view of that hesperetin, naringenin and apigenin all carry aryl hydroxyl groups in their chemical structures (cf. Fig. S1), it is not surprising that the three SULT1A1, SULT1A2, and SULT1A3 were found to display highest sulfating activity toward these three compounds.

2.2. Sulfation of hesperetin, naringenin and apigenin by human organ samples.

To investigate the presence of hesperetin-, naringenin- and/or apigenin-sulfating activity in human organs, enzymatic assays were performed using cytosols prepared from human lung, liver, small intestine, or kidney. Results obtained are compiled in Table 2. All four human organ cytosols tested showed sulfating activity towards hesperetin and naringenin. Among them, cytosols of small intestine and liver exhibited much higher activity than that of lung and kidney. With apigenin as a substrate, however, only liver and small intestine likely act as the major sites of sulfation for hesperetin, naringenin and apigenin. Considering that SULT1A1, SULT1A2, and SULT1A3 are abundantly expressed in the intestinal tract and the liver than in lung and kidney [Teubner et al., 2007; Riches et al., 2009], it is not surprising that higher sulfating activity in small intestine and liver cytosols than in lung and kidney cytosols. These findings are in accord with the previous study demonstrating that intestinal conjugation represents a more important component than hepatic conjugation for the metabolism of flavonoids in rats [Chen et al., 2003]. Interestingly, a number of studies have shown that flavonoids usually have low bioavailability due to their rapidly absorption followed by extensive first-pass metabolism [Walle et al., 2003]. It is possible that orally taken flavonoids may be first absorbed from the gastrointestinal tract and subjected to sulfation on site. Those that escape sulfation in the intestine may enter the circulation and reach the liver, where they may be sulfated by the SULTs therein. Collectively, these results provided a biochemical basis for the poor bioavailability of flavonoids.

2.3. Generation and release of [³⁵S]sulfated derivatives by HepG2 and Caco-2 cells labeled with [³⁵S]sulfate in the presence of hesperetin, naringenin and apigenin.

HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells were used to investigate whether sulfation of hesperetin, naringenin and apigenin may occur under metabolic conditions. HepG2 human hepatoma cells have been reported to express several SULT isoforms, including SULT1A1, SULT1A2, SULT1A3, SULT1E1, SULT2A1 [Westerink and Schoonen, 2007]; whereas Caco-2 human intestinal epithelial cells are known to express SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C2, SULT1C4, SULT2A1 [Meinl et al., 2008]. Confluent HepG2 and Caco-2 cells grown in individual wells of a 24-well plate, respectively, were labeled with [³⁵S]sulfate in sulfate-free medium supplemented with different concentrations (0, 5, 10, 25, 50, 100 μM) of hesperetin, naringenin and apigenin. TLC analysis of the labeling media collected at the end of an 18-hour labeling period revealed the generation and release of [³⁵S]sulfated hesperetin, naringenin and apigenin in a concentration-dependent manner by both HepG2 and Caco-2 cells (Fig. S2). These results therefore showed clearly that the sulfation of hesperetin, naringenin and apigenin may indeed occur in cultured cells under metabolic conditions. Radioactive spots corresponding to [³⁵S]sulfated hesperetin, naringenin and apigenin generated in the above-mentioned metabolic labeling experiment (Fig. S2) were cut out, eluted, and quantitatively determined by scintillation counting. Data obtained are compiled in Table S1.

3. Conclusions:

The current study bridged a gap in our understanding concerning the metabolism of flavonoids through sulfation. SULT1A1, SULT1A2, SULT1A3 and SULT1C4 were shown to be the major human SULTs responsible for the sulfation of hesperetin, naringenin and apigenin, while SULT1E1 is only involved in the sulfation of hesperetin, and SULT1B2 is responsible for sulfating hesperetin and naringenin. Cultured HepG2 and Caco-2 cells originating from liver and intestine, two major organs involved in first-pass metabolism, were used to demonstrate the occurrence of the sulfation of hesperetin, naringenin and apigenin under metabolic condition. Of the four human organ samples tested, liver and intestine cytosols showed strong activity towards hesperetin and naringenin, while lung and kidney cytosols indicated relatively low activity. In particular, apigenin was sulfated only in liver and intestine. Collectively, the results obtained implied that SULT-mediated sulfation plays a key role in the metabolism of hesperetin, naringenin and apigenin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ATP adenosine5'-triphosphate

DTT	dithiothreitol
FBS	fetal bovine serum
HEPES	N-2-hydroxylpiperazine-N'-2-ethanesulfonic acid
MEM	minimum essential medium
MES	morpholinoethanesulfonic acid
PAPS	3'-phosphoadenosine 5'-phosphosulfate
SULT	cytosolic sulfotransferase
TLC	thin-layer chromatography

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Table 1.Specific Activity of Human SULTs with Hesperetin, Naringenin and Apigenin as Substrates^a

SULT	Specific Activity (nmol/min/mg)		
	Hesperetin	Naringenin	Apigenin
1A1	44.17 ± 1.16	24.85 ± 0.65	10.67 ± 0.99
1A2	28.16 ± 0.38	17.51 ± 0.31	7.90 ± 0.32
1A3	29.80 ± 0.74	14.90 ± 0.15	6.38 ± 0.40
1B2	3.28 ± 0.96	0.56 ± 0.03	ND ^b
1C4	13.34 ± 0.49	11.99 ± 0.17	3.58 ± 0.09
1E1	1.51 ± 0.02	ND	ND

^aSpecific activity refers to nmol substrate sulfated/min/mg purified enzyme. The final concentration of the substrates tested was 10 µM. Data represent means ± SD derived from three experiments.

^bND refers to activity not detected.

Table 2.Specific Activities of Human Organ Cytosols with Hesperetin, Naringenin and Apigenin as Substrates^a

Substrate	Concentration	Specific Activity (pmol/min/mg)			
		Lung	Liver	Small intestine	Kidney
Hesperetin	10 μ M	23.31 $\pm$ 0.95	139.79 $\pm$ 6.87	458.26 $\pm$ 11.82	2.83 $\pm$ 0.40
	50 μ M	28.60 $\pm$ 4.68	181.73 $\pm$ 13.25	643.61 $\pm$ 45.12	5.66 $\pm$ 0.10
Naringenin	10 μ M	19.08 $\pm$ 1.50	135.32 $\pm$ 15.67	252.86 $\pm$ 13.22	2.77 $\pm$ 0.60
	50 μ M	26.53 $\pm$ 0.13	156.97 $\pm$ 17.94	354.21 $\pm$ 13.98	3.27 $\pm$ 1.27
Apigenin	10 μ M	ND ^b	20.87 $\pm$ 5.39	88.59 $\pm$ 1.07	ND ^b
	50 μ M	ND ^b	25.04 $\pm$ 2.20	113.24 $\pm$ 0.32	ND ^b

^aSpecific activity refers to pmol substrate sulfated/min/mg protein. Data represent mean $\pm$ SD derived from three experiments.^bND refers to activity not detected.