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Identification of a novel flavonoid glycoside sulfotransferase in *Arabidopsis thaliana*

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The discovery of sulfated flavonoids in plants suggests that sulfation may play a regulatory role in the physiological functions of flavonoids. Sulfation of flavonoids is mediated by cytosolic sulfotransferases (SULTs), which utilize 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the sulfate donor. A novel SULT from *Arabidopsis thaliana*, designated AtSULT202B7 (AGI code: At1g13420), was cloned and expressed in *Escherichia coli*. Using various compounds as potential substrates, we demonstrated, for the first time, that AtSULT202B7 displayed sulfating activity specific for flavonoids. Intriguingly, the recombinant enzyme preferred flavonoid glycosides (*e.g.* kaempferol-3-glucoside and quercetin-3-glucoside) rather than their aglycone counterparts. Among a series of hydroxyflavones tested, AtSULT202B7 showed the enzymatic activity only for 7-hydroxyflavone. pH-dependency study showed that the optimum pH was relatively low (pH 5.5) compared with those (pH 6.0–8.5) previously reported for other isoforms. Based on the comparison of high performance (pressure) liquid chromatography (HPLC) retention times between sulfated kaempferol and the deglycosylated product of sulfated kaempferol-3-glucoside, the sulfation site in sulfated kaempferol-3-glucoside appeared to be the hydroxyl group of the flavonoid skeleton. In addition, by using direct infusion mass spectrometry, it was found that the sulfated product had one sulfonate group within the molecule. These results indicated that AtSULT202B7 functions as a flavonoid glycoside 7-sulfotransferase.

Keywords: *Arabidopsis thaliana*/AtSULT202B7/flavonoid glycoside/sulfation/sulfotransferase.

Abbreviations: HPLC, high performance (pressure) liquid chromatography; IPTG, isopropyl β-D-thiogalactopyranoside; PAP, 3′-phosphoadenosine 5′-phosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PCR, polymerase chain reaction; TLC, thin-layer chromatography; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; SULT, cytosolic sulfotransferase.

Flavonoids are a group of secondary metabolites distributed in a wide range of plant species. They share a common phenyl benzopyrone structure and are divided into major subclasses (*e.g.* flavanone, flavonol, flavone, isoflavone and anthocyanidin) based on the numbers and positions of the hydroxyl group and the C-ring structure. Flavonoids have been implicated in a variety of physiological functions, *e.g.* provision of colors attractive to pollinators (*1*); protection of the plant body from external stress such as fungal infection and UV irradiation (*2*); communication with the symbiont *Rhizobia* (*3*); and influence in the transport of the plant hormone, auxin (*4*). To date, nearly 9000 structural variants of flavonoids have been reported (*5*). In different plants, flavonoids occur as glycosides (*e.g.* glucoside, galactoside, rhamnoside and arabinoside), which, except for flavanols such as catechins and proanthocyanidins, are generated upon glycosylation by uridine-diphosphate glucose glycosyltransferases (*6*). Glycosylation increases the solubility and the stability of flavonoids, and reduces their reactivity (*7*). In addition to glycosylation, flavonoids are known to undergo sulfation conjugation in at least 32 families of plants (*8*). Sulfation conjugation has been extensively studied in humans, but not in plants. Sulfation is catalyzed by the so-called cytosolic sulfotransferases (SULTs). The universal sulfate donor for SULT-mediated sulfation is 3′-phosphoadenosine 5′-phosphosulfate (PAPS). During the sulfation reaction, the sulfonate group (SO_3^-) is transferred from PAPS to an acceptor hydroxyl or amino group of the substrate compounds with the concomitant formation of 3′-phosphoadenosine 5′-phosphate (PAP). In humans, SULT-mediated sulfation is one of the major phase II conjugation reactions, which plays an important role in the metabolism and regulation of key endogenous compounds and the detoxification of xenobiotics (*9*). Interestingly, SULT homologs have been shown to be present in a variety of plant species as indicated by a large number of plant SULT protein sequences deposited in different databases. However, their physiological functions have remained poorly characterized. In *Arabidopsis thaliana*, a popular

model plant species, there are 17 SULT genes (*10*). To date, only a few *A. thaliana* SULT isoforms have been isolated and biochemically characterized.

In this communication, we report the identification and characterization of a novel *A. thaliana* sulfotransferase, designated AtSULT202B7 (AGI code: At1g13420). The enzymatic activity of recombinant AtSULT202B7 toward a major flavonol and its glycosides was tested. A systematic analysis of the optimum pH and kinetics parameters toward flavonols and their glycosides was performed. To the best of our knowledge, this is the first report on a sulfotransferase capable of catalyzing the sulfation of flavonoid glycosides in *A. thaliana*.

Materials and Methods

Materials

Kaempferol, quercetin and glucose were purchased from Wako Pure Chemical Industries. Quercetin-3-glucoside, 5-hydroxyflavone, arbutin, salicin and 3-hydroxyflavone were products of Sigma-Aldrich Co. LLC. Quercetin-3-galactoside, quercetin-3-rutinoside, kaempferol-3-glucoside, kaempferol-7-glucoside and kaempferol-3-robinoside-7-rhamnoside were from Extrasynthese. 7-Hydroxyflavone was purchased from Tokyo Kasei Kogyo Co., Ltd. Quercetin-7-glucoside was a product of Apin Chemical Ltd. Quercetin-3-rhamnoside, 3′-hydroxyflavone and 4′-hydroxyflavone were obtained from Indofine Chemical. pBluescript II SK (+) vector, XL1-Blue MRF' and BL21 *Escherichia coli* host strain were obtained from Stratagene. pGEX-4 T-1 prokaryotic GST fusion vectors and glutathione sepharose 4B were from GE Healthcare Biosciences. Cellulose thin-layer chromatography (TLC) plates were products of Merck. All other chemicals were of the highest grade commercially available.

Molecular cloning of AtSULT202B7

Arabidopsis thaliana ecotypes Col-0 were grown with a 16-h photoperiod at 22°C with 50–60% humidity. Two-week-old *Arabidopsis* seedlings were frozen in liquid nitrogen and homogenized in a TRIzol RNA Isolation Reagent (Life Technology), according to the manufacturer's instructions. With 1 μg of the isolated total RNA as the template and oligo (dT) as the primer, first-strand cDNA was synthesized using the First-Strand cDNA Synthesis Kit (TOYOBO). Polymerase chain reaction (PCR) was carried out in a 20 μl reaction mixture using AtSULT202B7-sense (5′-CGCGGATC CATGGGTGAGAAAGATATTCCA-3′) and AtSULT202B7-antisense (5′-CGGAATTCCTACAATTTCAAACCAGAGCCT-3′) primers under the action of KOD-Plus-Neo DNA polymerase (TOYOBO). The PCR conditions were 94°C for 2 min, followed by 35 cycles of 10 s at 98°C, 30 s at 55°C, 40 s at 68°C and a final incubation at 68°C for 7 min. The amplified product was restricted using BamHI and EcoRI (TOYOBO), subcloned into pBluescript II SK (+), and transformed into *E. coli* XL1-Blue MRF'. To verify its authenticity, the cDNA insert was subjected to nucleotide sequencing. Upon verification, the insert was subcloned into pGEX-4 T-1 prokaryotic expression vector.

Bacterial expression and purification of recombinant AtSULT202B7

pGEX-4 T-1 harbouring the cloned AtSULT202B7 cDNA (GenBank ID: NP_172799) was transformed into competent *E. coli* BL21 cells. Transformed BL21 cells were grown to $OD_{600nm} = \sim 0.3$ in 100 mL LB medium supplemented with 100 μg/mL ampicillin, and induced with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG). After a 12-h induction at 24°C, the cells were collected by centrifugation and homogenized in 15 mL of ice-cold lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl and 1 mM ethylenediaminetetraacetic acid) using a French Press (Ohtake Works Co. Ltd.). The crude homogenate was subjected to centrifugation at 20,400 × *g* for 15 min at 4°C. The supernatant collected was fractionated using 0.5 mL of glutathione Sepharose 4B, and the bound GST fusion protein was treated with 0.2 mL of a thrombin digestion buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl and 2.5 mM $CaCl_2$) containing 5 units/mL bovine thrombin. Following a 2-h incubation period at 4°C with constant agitation, the preparation was subjected to centrifugation, and the supernatant containing purified recombinant SULT was collected and used in the enzymatic assay.

Enzymatic assay

Sulfating activity of AtSULT202B7 was assayed using ^{35}S-PAPS as the sulfate donor. The standard assay mixture, with a final volume of 25 μL, contained 50 mM sodium phosphate buffer, pH 7.5, 0.2 μM ^{35}S-PAPS (45 Ci/mmol) and 100 μM substrate. The reaction was started by the addition of the enzyme, allowed to proceed for 20 min at 22°C, and terminated by heating at 98°C for 3 min. The precipitates formed were removed by centrifugation, and the supernatant was subjected to the analysis of the ^{35}S-sulfated product using a previously developed TLC separation procedure (*11*) with *n*-butanol/isopropanol/formic acid/water (3:1:1:1 by volume) or *n*-butanol/acetic acid/water (3:1:1 by volume) as the solvent system. Afterwards, the plate was air-dried and analyzed using a Fluoro Image Analyzer FLA-3000 (Fujifilm). To examine the pH-dependence, different buffers (50 mM sodium acetate buffer at pH 4.0–6.0 and 50 mM sodium phosphate buffer at 6.5–8.0) were used in the reaction mixtures. For the kinetic studies on the sulfation of flavonoids, substrates with different concentrations ranging from 0.01 to 40 μM were used. Data obtained were processed using the Excel program to generate the best fitting trendline for the Lineweaver–Burk plots. No enzyme inhibition was observed over the range of the PAPS and flavonoids concentration used.

Identification of sulfated kaempferol-3-glucoside by reverse phase high performance (pressure) liquid chromatography followed by direct infusion mass spectrometry

The sulfation reaction was performed in a reaction mixture (with a final volume of 250 μl) containing 50 mM sodium acetate buffer, a pH of 5.5, 100 μM PAPS, 20 μM kaempferol or kaempferol-3-glucoside and 25 μg of purified AtSULT202B7. The reaction was started by the addition of the enzyme, allowed to proceed for 12 h at 22°C, and terminated by heating at 98°C for 5 min. The precipitates formed were removed by centrifugation, and the supernatant was subjected to the analysis using a Shimadzu Prominence HPLC system consisting of a photodiode array detector and the LC Solution software was used for all high performance (pressure) liquid chromatography (HPLC) analysis. 5 μm Capcell PAK C18 MG column (250 × 4.6 mm; SHISEIDO), and a gradient elution of acetonitrile and ultrapure water were used for the HPLC separations. For all analyses, the injection volume was 40 μL; the flow rate was 1 mL/min; and the controlled oven temperature was 40°C. Fraction containing sulfated kaempferol-3-glucoside was lyophilized and dissolved in dimethylsulfoxide. Deglucosylation reaction was conducted in a reaction mixture (with a final volume of 250 μl) containing 100 mM sodium acetate buffer, pH 5.0, 8 units/mL of β-glucosidase from sweet almond and 10 μL of the redissolved sulfated kaempferol-3-glucoside. The reaction was started by the addition of the enzyme, allowed to proceed for 24 h at 37°C, and terminated by heating at 98°C for 5 min. The precipitates formed were removed by centrifugation, and the supernatant was subjected to the earlier described HPLC analysis. The sulfated kaempferol-3-glucoside dissolved in methanol was analyzed by Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher scientific) with a heated electrospray ionization source through direct infusion using a syringe pump. These data were acquired using Targeted-MS^2 scan event. Typical mass spectrometric conditions were as follows: polarity, negative ionization mode; spray voltage, 3.5 kV; sheath gas flow rate, 6; auxillary gas, 0; sweep gas, 0; heated capillary temperature, 320°C. The resolution was set at 140,000. The AGC target was 2E5. The maximum ion injection time was 100 ms. The normalized collision energy was 20%. The raw data files were analyzed using Qual Browser software in Xcalibur (Thermo fisher scientific).

Miscellaneous methods

To prepare the solutions for use in the enzymatic assay, all substrate compounds were dissolved in dimethylsulfoxide. ^{35}S-PAPS (45 Ci/mmol) was synthesized from ATP and ^{35}S-sulfate using recombinant

human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase, as previously described (*12*). Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on 10% polyacrylamidegels using the method of Laemmli (*13*). Protein determination was performed based on Lowry's method, with bovine serum albumin as the standard (*14*).

Results and Discussion

Cloning of AtSULT202B7

For the *A. thaliana* genome, the At1g13420 (AGI code) gene was named AtSULT202B7 according to a previously reported nomenclature system (*15*, *16*). The deduced amino acid sequence of AtSULT202B7 was aligned with AtSULT202B1, a flavonol-7 sulfotransferase (*17*), and AtSULT202B5, an uncharacterized sulfotransferase, using the Clustal W program. Box shade software (http://www.ch.embnet.org/software/BOX_form.html) was used to show identical residues in the alignment (Fig. 1). The four highly conserved regions containing the PAPS binding and catalytic sites (*18–20*) were identified in the AtSULT202B7 sequence. Using GENETYX-MAC version 11.1.0, AtSULT202B7 was shown to display 66.6% and 73.0% amino acid sequence identities to AtSULT202B1 and AtSULT202B5, respectively. The open reading frames of AtSULT202B7 sequence was PCR-amplified using gene-specific primers (cf. Materials and Methods). The PCR product was cloned into the pBluescript II SK (+) vector and sequenced. Sequence data obtained completely matched with that shown for the GenBank accession number NP_172799 (At1g13420). The coding sequence of AtSULT202B7 was subsequently subcloned into pGEX-4T-1, a prokaryotic expression vector, for the expression of recombinant enzymes in *E. coli*. As shown in Fig. 2, a GST fusion protein (~64 kDa) was expressed upon induction with IPTG, and recombinant AtSULT202B7 was purified to near homogeneity. The molecular weight of AtSULT202B7 was calculated using the online program Protparam (http://web.expasy.org/protparam/) and was determined to have an expected molecular mass of 37.7 kDa. In line with the predicted molecular mass, purified AtSULT202B5, upon SDS–PAGE, migrated at 40.7 kDa position (data not shown).

Determination of pH optimum

In higher plants, flavonoids commonly exist as glycoside such as glucosides (Fig. 3). In an initial experiment, kaempferol-3-glucoside and quercetin-3-glucoside were tested as substrates for AtSULT202B7 at pH 7.5. AtSULT202B7 showed strong activities (138.6 and 80.9 pmol/min/mg enzyme, respectively) toward kaempferol-3-glucoside and quercetin-3-glucoside. Next, the pH dependence of the activity of AtSULT202B7 was determined using two substrates,

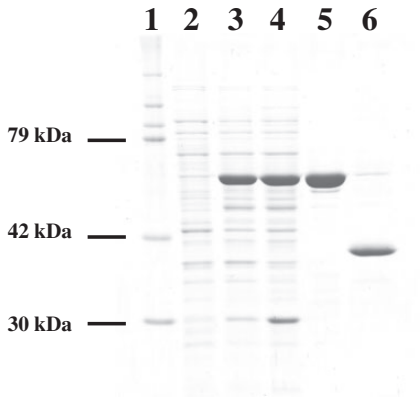


Fig. 2 SDS gel electrophoretic pattern of recombinant AtSULT202B7 at different stages during purification. Samples were subjected to SDS–PAGE, followed by Coomassie blue staining. Lane 1, molecular weight markers; lane 2, BL21 *Escherichia coli* homogenate prior to IPTG induction; lane 3, BL21 *E. coli* homogenate after IPTG induction; lane 4, cytosolic fraction of IPTG-induced BL21 *E. coli* homogenate; lane 5, AtSULT202B7-GST-fusion protein before thrombin cleavage; lane 6, purified AtSULT202B7 after treatment of thrombin.

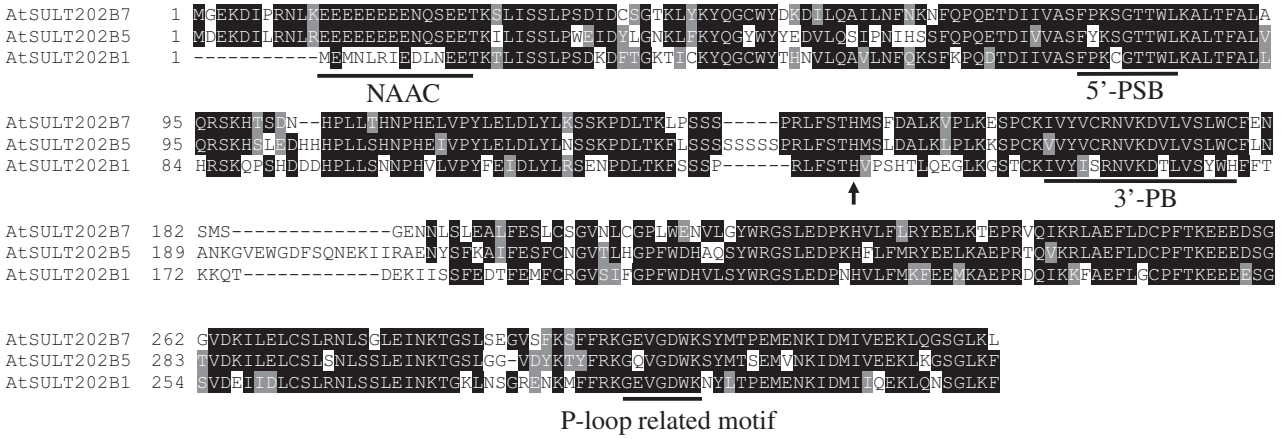
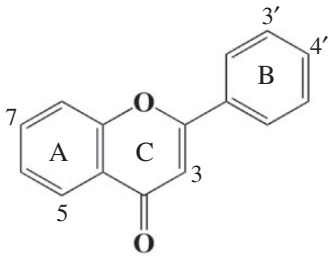


Fig. 1 Amino acid sequence comparison of AtSULT202B7, AtSULT202B5 and AtSULT202B1. Identical residues conserved among at least 2 of the 3 enzymes are indicated by black background, and similar residues are indicated in grey. The NAAC (the N-terminal acidic amino acid cluster), which might act as a potencial sorting determinant, is underlined. The 5′-PSB loop (including the conserved lysine residue in the N terminal), which interacts with the 5′-phosphate of PAP, and the 3′-PB motif, for the binding of the 3′-phosphate of PAP, are underlined. The conserved P-loop related motif (GXXGXXK) is also underlined. The catalytic histidine residue, which is highly conserved in almost all known SULTs, is indicated by an arrow.

kaempferol-3-glucoside and quercetin-3-glucoside. AtSULT202B7 displayed the highest sulfating activity at pH 5.5 for these two substrates, and the activity started decreasing at higher pHs (Fig. 4). Previous studies have shown that flavonol sulfotransferases derived from *Flaveria* species displayed optimum pH ranging 6.0–8.5 (*21*). Considering that the vacuolar pH of plant cells is about 5.0–5.5 (*22*) and that the flavonol glycosides accumulates mainly in the vacuole (*23*), it is possible that AtSULT202B7 might somehow be transported into this acidic organelle and sulfate the flavonol glycosides locally as substrates. No reported vacuole sorting determinant, however, could be discerned in the amino acid sequence of AtSULT202B7. Using the computer program PSORT (http://psort.hgc.jp/), AtSULT202B7 has been reported as a cytoplasmic protein (*10*), while its exact subcellular location still remains unknown. Further examination of the amino acid sequence of AtSULT202B7 revealed an acidic amino acid cluster located in its N-terminal region of AtSULT202B7 (Fig. 1). It may be worthwhile mentioning that previous studies have reported that acidic amino acid cluster might serve as a basolateral sorting signal in MDCK cell (*24*). The N-terminal acidic amino acid cluster in AtSULT202B7, therefore, could be proposed to function as a novel sorting determinant for a certain organelle in the cells of *A. thaliana*. To this end, immunocytochemical analysis using specific antibody against AtSULT202B7 will be required to unequivocally identify the subcellular location of AtSULT202B7.



Compound	C3	C5	C7	C3′	C4′
Kaempferol-3-glucoside	Glucose	OH	OH	H	OH
Quercetin-3-glucoside	Glucose	OH	OH	OH	OH
Kaempferol	OH	OH	OH	H	OH
Quercetin	OH	OH	OH	OH	OH
3-Hydroxyflavone	OH	H	H	H	H
7-Hydroxyflavone	H	H	OH	H	H

Fig. 3 A chemical structure of some representative flavonoids used as substrates for this study.

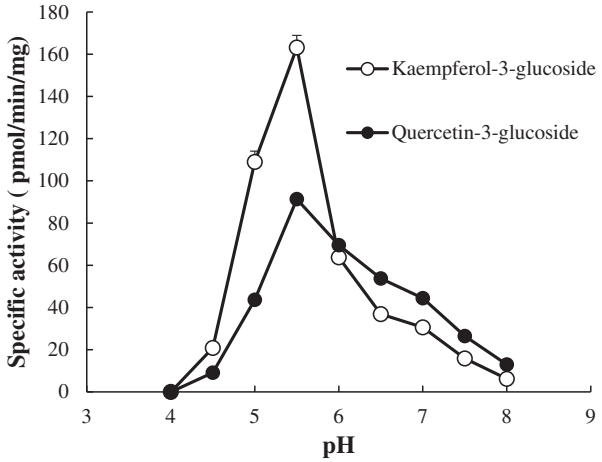


Fig. 4 pH dependency of the sulfating activity of AtSULT202B7 with flavonols and flavonol glucosides as substrates. The enzymatic assays with 10 μM of substrate compounds were carried out under standard assay conditions using different buffer systems.

Substrate specificity of AtSULT202B7 for a variety of flavonoids and related compounds

Under optimum pH conditions, the enzymatic activity of AtSULT202B7 toward a variety of flavonoids and related compounds were examined. As shown in Table I, AtSULT202B7 displayed considerably higher activity toward a wide range of flavonol glycosides (*e.g.* quercetin-3-glucoside, quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-rhamnoside and kaempferol-3-glucoside) than their aglycone counterparts. These results suggested that AtSULT202B7 has no strict sugar selectivity in the sulfation of flavonoid glycosides. Interestingly, no activity was detected with flavonols having a sugar at position 7 (*e.g.* quercetin-7-glucoside, kaempferol-7-glucoside and kaempferol-3-robinoside-7-rhamnoside), suggesting that AtSULT202B7 may sulfate the 7-hydroxyl group of flavonols in a position-specific manner. This possibility was confirmed in a subsequent experiment using five kinds of hydroxyflavones that contain only one hydroxyl group at 3, 5, 7, 3′ and 4′ as substrates. Of the five, only 7-hydroxyflavone was sulfated. These results supported the notion that AtSULT202B7 was active toward only flavonoids with a hydroxyl group at position 7. Moreover, no sulfation products of glucose and phenolic plant glucosides, such as arbutin and salicin, were detected. Collectively, these results suggested

Table I. Specific activities of AtSULT202B7 with various flavonoids as substrates.

Substrate compound	Specific activity (pmol/min/mg)
Kaempferol	63.3 ± 3.4
Kaempferol-3-glucoside	343.8 ± 8.5
Kaempferol-7-glucoside	N.D.
Kaempfrol-3-robinoside-7-rhamnoside	N.D.
Quercetin	47.8 ± 3.2
Quercetin-3-glucoside	311.7 ± 4.5
Quercetin-7-glucoside	N.D.
Quercetin-3-galactoside	235.0 ± 3.6
Quercetin-3-rutinoside	183.1 ± 4.5
Quercetin-3-rhamnoside	153.8 ± 3.6
3-Hydroxyflavone	N.D.
5-Hydroxyflavone	N.D.
3′-Hydroxyflavone	N.D.
4′-Hydroxyflavone	N.D.
7-Hydroxyflavone	16.9 ± 1.5
Salicin	N.D.
Arbutin	N.D.
Glucose	N.D.
UDP-glucose	N.D.

Specific activity refers to pmol of sulfated product formed/min/mg enzyme. Data shown represent means ± S.D. from three determinations. N.D. refers to activity not detected (<5.0 pmol/min/mg).

that sulfation by AtSULT202B7 requires the presence of a flavone backbone in substrate compounds. It is interesting to note that despite the high amino acid sequence similarity between AtSULT202B5 and AtSULT202B7, AtSULT202B5 showed no activity toward a variety of flavonoids, including its glycosides. Other phenolic and steroidal compounds, such as phytohormones and brassinosteroids, were also tested as substrates for AtSULT202B7. No activity, however, was detected.

Determination of kinetic parameters for AtSULT202B7-mediated sulfation of flavonols and flavonol-3-glucosides

Kinetic parameters (V_{max}, K_m and V_{max}/K_m) for the sulfation of kaempferol, kaempferol-3-glucoside, quercetin, or quercetin-3-glucoside by AtSULT202B7 were determined. The results compiled in Table II indicate that the apparent K_m for kaempferol was 27.6 μM, and that the V_{max} value was 209.5 pmol/min/mg of enzyme.

Table II. Kinetic constants of the sulfation of flavonols and flavonol glucosides by AtSULT202B7.

	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m
Kaempferol	27.6 ± 2.7	209.5 ± 16.4	7.6
Kaempferol-3-glucoside	14.4 ± 2.7	592.1 ± 57.0	41.1
Quercetin	42.1 ± 8.1	224.1 ± 25.1	5.3
Quercetin-3-glucoside	18.1 ± 1.9	418.2 ± 31.5	23.1
PAPS	0.4 ± 0.1	495.4 ± 43.4	1238.5

The kinetic parameters of PAPS were examined with 10 μM of kaempferol 3-glucoside. Data shown represent means ± S.D. from three determinations.

The K_m value for kaempferol-3-glucoside was 14.4 μM, and the V_{max} was 592.1 pmol/min/mg of enzyme. Based on these results, the V_{max}/K_m for kaempferol-3-glucoside was about five times higher than that for its aglycone counterpart. Similar results were also found with quercetin and its glucoside as substrates. These findings suggested that AtSULT202B7 sulfates flavonol-3-glucosides more efficiently than flavonol aglycones. The K_m values for PAPS, determined with fixed concentration of kaempferol-3-glucoside, was 0.4 μM. which is within the range of K_m values (0.2–0.4 μM) previously reported for other flavonoid sulfotransferases (*21*).

HPLC detection and mass spectrometry of sulfated kaempferol 3-glucoside

To further determine the structure of sulfated flavonol glucoside, deglucosylated products of sulfated kaempferol-3-glucoside, generated upon treatment with β-glucosidase, were subjected to HPLC analysis. The retention time and the wavelength of maximum absorption (λ_{max}) of the deglucosylated products were found to be almost identical to those of sulfated kaempferol (Fig. 5). These results suggested that AtSULT202B7 sulfates the hydroxyl group of the flavone backbone. To date, 3-sulfatoglucoside and 3-sulfatorhamnoside of flavonols have been detected in some plant species (*8*). To clarify whether the glucose moiety of kaempferol-3-glucoside can indeed be sulfated by AtSULT202B7, we performed mass spectrometry of sulfated kaempferol-3-glucoside in negative ion mode. The parent ion, the highest relative abundance of ion in full scan mode was at m/z 527.05. As shown in Fig. 6, further selected ion monitoring of the parent ion and the following high-energy

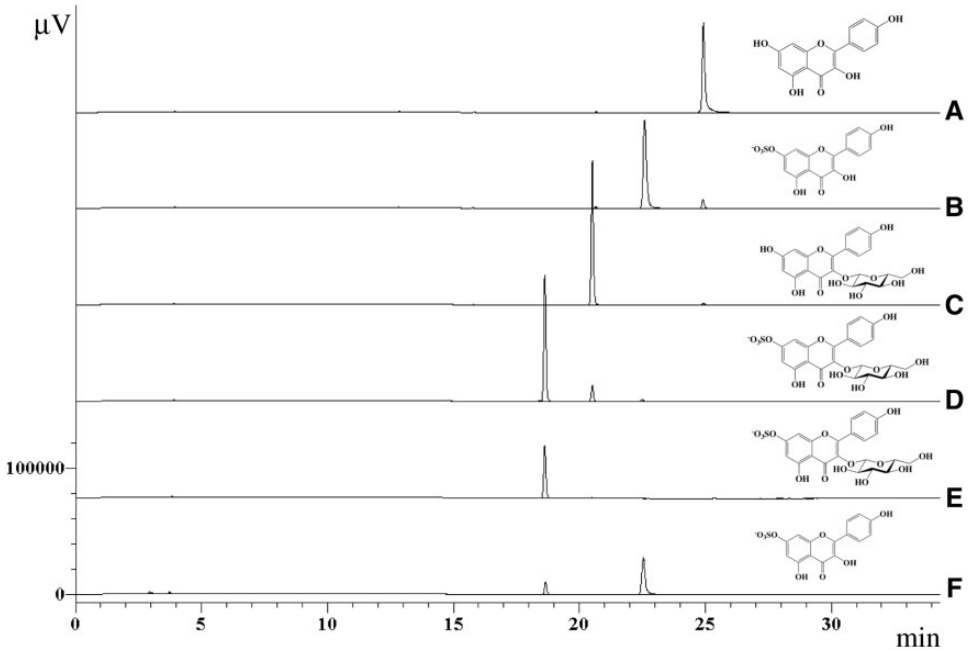


Fig. 5 HPLC detection of sulfated kaempferol-3-glucoside. (A) Kaempferol; (B) sulfated kaempferol generated using AtSULT202B7; (C) kaempferol-3-glucoside; (D) sulfated kaempferol-3-glucoside generated using AtSULT202B7; (E) sulfated kaempferol-3-glucoside; (F) deglucosylated products of sulfated kaempferol-3-glucoside generated using β-glucosidase.

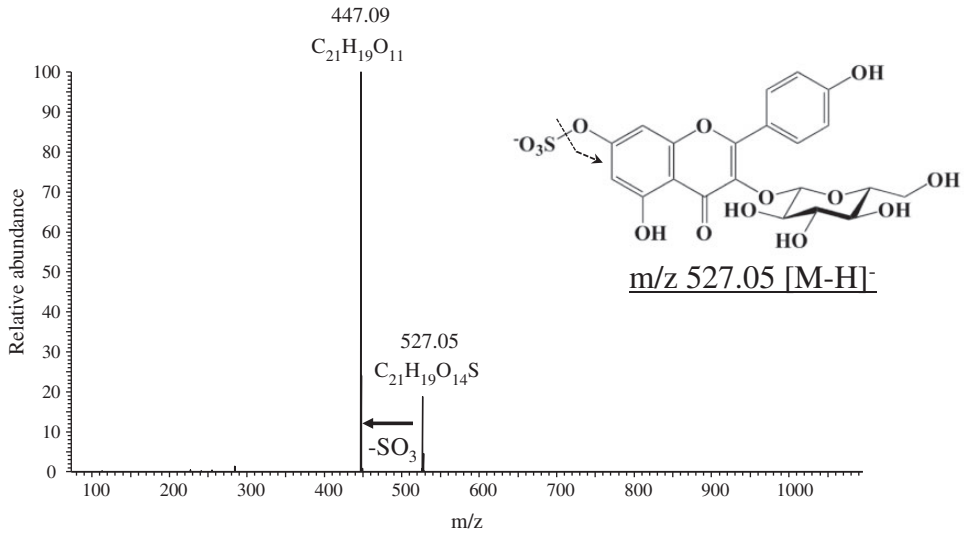


Fig. 6 MS2 spectrum of the sulfated kaempferol-3-glucoside. MS2 data were obtained from the 527.05 m/z ion as the precursor for high energy collisional dissociation.

collision dissociation fragmentation of the parent ion produced authentic kaempferol-3-glucoside lacking one SO_3^- group (m/z 79.96) at m/z 447.09. These results strongly suggests that sulfated kaempferol-3-glucoside by AtSULT202B7 contains no sulfonate group in glucose moiety.

To summarize, we have cloned, expressed, purified and characterized a novel *A. thaliana* sulfotransferase, AtSULT202B7, in this study. AtSULT202B7 was shown to exhibit higher affinity toward flavonol glucosides than its aglycone derivative. AtSULT202B7 displayed sulfating activity only for 7-hydroxyflavone, but not for flavonol-7-glucosides. Moreover, the deglucosylated product of sulfated kaempferol-3-glucoside is more likely to be the sulfated kaempferol, and the product lost the sulfonate group to become kaempferol-3-glucoside, suggesting that AtSULT202B7 might be a flavonol glucoside 7-sulfotransferase. The high content of flavonoid glycosides in plants and the high specific activity of AtSULT202B7 for these compounds might imply the presence of sulfated flavonoid glycosides in plants. Attempting to detect sulfated flavonoid glycoside *in vivo*, we performed an Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis of the methanol extracts of mature *A. thaliana* plants. Repeated experiments, however, failed to detect the presence of sulfated flavonoid glycoside. Moreover, extensive metabolomic database search yielded no evidence for the existence of sulfated flavonoid glycoside in *A. thaliana*. It is therefore likely that the abundance of those compounds may be extremely low, or that the sulfated flavonoid glycosides might be produced only under certain conditions, such as at a particular developmental stage or under environmental stress. To date, it has been reported that AtSULT202B7 is expressed predominantly in the root under a publicly available microarray expression data bank (http://www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). Flavonoids are involved in the polar auxin transport in roots (*25*), suggesting that SULT202B7 might regulate the growth and development of the root via flavonoid glycosides sulfation.

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Conflict of interest

None declared.

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