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# A Novel Sulfotransferase Abundantly Expressed in the Dauer Larvae of *Caenorhabditis elegans*

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**We have isolated a gene (clone Y113G7A.11) from *Caenorhabditis elegans* (*C. elegans*), that we have designated as ceST1, and which is the only member of the cytosolic sulfotransferase (SULT) gene family present in the genome of this organism. We identified the SULT motifs of ceST1 based upon their deduced amino acid sequence, and subsequently expressed the ceST1 cDNA in *Escherichia coli* and characterized its enzymatic properties. The recombinant protein showed sulfation activity for 4-nitrophenol and 2-naphthol substrates, but did not catalyze the sulfation of either monoamines or hydroxysteroids. Another compound sulfated by ceST1 is bisphenol A, which is known to stimulate germ cell proliferation in *C. elegans*. SULT activity was not detected in the cytosol of *C. elegans*, probably due to heat labile inhibitors. The ceST1 protein was detectable in the cytosol of *C. elegans* using anti-sera raised against recombinant ceST1, and transcripts could also be detected throughout the developmental stages. Moreover, high levels of ceST1 expression were evident at both the embryonic and adult stages and were augmented in dauer larva. These findings suggest that this sulfotransferase either forms part of a defence system against xenobiotics or regulates germ cell proliferation in *C. elegans*.**

**Key words: bisphenol A, *C. elegans*, dauer, SULT.**

Abbreviations: DDBJ, DNA data bank of Japan; NGM, nematode growth medium; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PBS, phosphate buffered saline; RT-PCR, reverse transcriptase–polymerase chain reaction; SULT, cytosolic sulfotransferase; UniProt, Universal Protein Resource

Sulfation has evolved as a key component of xenobiotic metabolism and chemical defence mechanisms, but also has important roles in modulating the biological activity of numerous potent endogenous chemicals including iodothyronines, steroids and catecholamines (*1–3*). Sulfation is catalyzed by the members of the cytosolic sulfotransferase (SULT) enzyme family, which in mammals have been classified into six families (*4*). Among these, SULT1 and SULT2 have been the most extensively characterized. SULT1 family members catalyze the translocation of sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to phenolic substances. SULT2 members, on the other hand, catalyze the conjugation of hydroxysteroids. Recent reports have identified and characterised novel SULTs from a variety of non-mammalian vertebrates (*5–7*). Various SULT genes are also registered in the genome databases of invertebrates, but their enzymatic characteristics remain to be elucidated, with the exception of SULT101A1. SULT101A1 was originally purified from *Spodoptera frugiperda* (*8*) and designated as a retinol dehydratase based on its enzymatic activity. This gene was later classified as a SULT family member by amino acid sequence alignment.

*C. elegans* is one of the most widely used genetic model organisms and has only one SULT homologue in its genome, in contrast to the human genome which has 17 SULT genes (*4*). By BLASTP search performed against Wormpep with human SULT2B1b as the query sequence, we identified only one SULT homologue, denoted as Y113G7A.11, and we designated this clone as ceST1. Although the deduced amino acid sequence of the ceST1 gene contains an identifying motif (Pfam domain PF00685), its enzymatic activity has not previously been demonstrated. Hence, we have generated a recombinant protein and characterized its catalytic properties, and also investigated its expression in the various developmental stages of this worm.

## EXPERIMENTAL PROCEDURES

*Materials*—[$^{35}$S]PAPS was purchased from PerkinElmer Life Sciences (Boston, MA). Cold PAPS was prepared as previously described (*9*). The prokaryotic expression vector, pGEX6P-1, PreScission protease, glutathione Sepharose 4B, Sephacryl S-200 columns and the ECL Plus Western Blotting Detection System were obtained from Amersham Biosciences (Uppsala, Sweden). DH-5α competent cells were purchased from TOYOBO (Tokyo, Japan); BL21 competent cells were obtained from the Novagen brand of Merck KGaA (Darmstadt, Germany). All other materials used in the nucleic acid experiments were purchased from Takara Shuzo Co. (Kyoto, Japan). The ceST1 antiserum was produced by Ark Resource (Kumamoto, Japan), and the additional materials used in other experiments were obtained from Wako Chemicals (Osaka, Japan). The N2 and *daf-2 (e1370)* strains were

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obtained from the Caenorhabditis Genetics Center and maintained on nematode growth medium (NGM) agar plates with *E. coli* OP50 as a food source (*10*).

*Bacterial Expression of ceST1 and Its Purification*—Total RNA was isolated from worms by acid guanidinium thiocyanate phenol—chloroform extraction with ISOGEN (Nippon Gene, Tokyo, Japan). The first strand of ceST1 cDNA was synthesized from 1 μg of total RNA using 1 unit of M-MLV reverse transcriptase with oligo(dT) primers according to the manufacturer's protocol (Stratagene Alameda, CA). The coding sequence was then amplified by PCR with primers containing *Bam*HI and *Not*I consensus sites to facilitate cloning. The primers used were 5′-CTGGGATCCATGACCCCGAAGACCCCA-AAGACC-3′ (forward) and 5′-ATGCGGCCGCCTACTCA-GCGAAAGTGGACAAATC-3′ (reverse). The amplified fragment was then digested with *Bam*HI and *Not*I and ligated into the prokaryotic expression vector pGEX6P-1. The resulting construct was amplified in *E. coli* DH5-α cells and purified with a QIAGEN plasmid midi kit (QIAGEN, Valencia, CA). The ceST1 cDNA insert was sequenced by the CEQ2000 Dye Terminator Cycle Sequencing with a Quick Start Kit (Beckman Coulter, Krefeld, Germany).

To generate recombinant SULT, the pGEX6P-1 vector expressing ceST1 was transformed into the *E. coli* BL21 strain and cultivated in Luria-Bertani broth containing 50 μg/ml ampicillin for 16 h at 37°C. A 24 ml aliquot of the culture fluid was then inoculated into 1,200 ml of M9-minimal medium with 1% glucose, 10% sucrose, 0.1% betaine and 50 μg/ml ampicillin. The bacterial cultures were grown at 37°C to an $OD_{600}$ of 0.4 and stimulated with 0.5 mM IPTG for 4 h at 20°C. The cells were harvested by centrifugation at 6,000 × *g* for 10 min at 4°C and resuspended in 40 ml of ice-cold PBS with 1.5 mM DTT. After sonication, the lysate was centrifuged at 12,000 × *g* for 10 min at 4°C. The resulting supernatant was then filtered (pore size 0.45 μm) and purified by glutathione Sepharose 4B and Sephacryl S-200 column chromatography. The purification procedures for recombinant ceST1 were next carried out at 4°C. The soluble fraction containing recombinant ceST1 was loaded onto a glutathione Sepharose 4B column that had been preequilibrated with PBS containing 1.5 mM DTT. The column was washed with the same buffer, and recombinant proteins were eluted by digestion with PreScission protease according to the manufacturer's protocol. The eluate was then loaded onto a Sephacryl S-200 high load column, preequilibrated with buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1.5 mM DTT, 150 mM NaCl and 10% glycerol.

*SULT Activity Assay*—The SULT activity of the purified recombinant ceST1 was determined using [$^{35}$S]PAPS as the sulfate donor and the indicated compounds as sulfate acceptors according to the procedure of Foldes and Meek (*11*) with slight modifications. Briefly, under typical assay conditions, the reaction mixture (250 μl) contained 20 mM phosphate buffer, pH 6.8, 8.0 μM [$^{35}$S]PAPS (0.1 μCi), 10 mM $MgCl_2$, 1.5 mM DTT, purified protein (0.1 μg) and various concentrations of acceptors. The mixture was incubated at 25°C for 15 min and the reaction was stopped by the addition of 100 μl of an equal mixture of 0.1 M barium acetate and barium hydroxide. Fifty microliters of 0.1 M zinc sulfate was then added and the mixture was centrifuged at 1,200 × *g* for 5 min. Aliquots (300 μl) of the supernatants were transferred to new microcentrifuge tubes, and 50 μl of an equal volume of 0.1 M barium hydroxide and 0.1 M zinc sulfate was added to each tube. The mixtures were centrifuged at 13,000 × *g* for 5 min, and the radioactivity in 300 μl aliquots of the resulting supernatants were measured by scintillation spectrometry. One unit of enzyme activity was defined as the amount required to transfer 1 pmol of sulfate/min and activity is expressed as pmol/min.

*Cultivation of Staged Worms*—We prepared staged worms as follows. Alkaline hypochlorite purified embryos were inoculated onto NGM agar plates and the plate cultures were incubated at 20°C for the following times: L1—2, 22 h, L2—3, 48 h, L3—4, 60 h, L4-adult 72 h. Worms were then washed off in M9 buffer and pelleted by centrifugation for 1 min in a microfuge. Following freezing at —80°C, total RNA was isolated and cDNA was synthesized as described above.

A *daf-2 (e1370)* mutant culture was synchronized by hatching alkaline hypochlorite purified embryos in the absence of food. The resulting synchronously arrested L1 larvae were inoculated onto NGM agar plates. The plate cultures were then incubated at 25°C for 72 h to induce dauer formation. L3—4 control larvae were incubated at 15°C for 72 h and the following 25°C cultivation was performed in order to suppress the DAF-2 signal, without forming dauer larvae.

*Real-Time RT-PCR*—One half microliter of cDNA was added to a 24.5 μl PCR reaction mixture consisting of 12.5 μl of SYBRgreen mix including AmpliTaq gold and cyber green (Applied Biosystem, Warrington, UK), and 0.25 μM of both sense and antisense primers. The specific primers used to amplify the target cDNAs were as follows: *ama-1* (434 bp), 5′-gcattgtctcacgcgttcag-3′ forward, 5′-ttct-tccttctccgctgctc-3′ reverse, *ceST1* (552 bp), 5′-cgtcagaaaccc-caaagactg-3′ forward, 5′-tgctcctcctcttcttcttcc-3′ reverse. The cycling conditions were 30 s at 94°C, 30 s at 57°C and 1 min at 72°C (40 cycles). These reactions were followed in real time using the ABI PRISM 7700 Sequence Detection System. The relative amount of target was calculated using the 2-CT method (CT: refers to the threshold cycle for target amplification) (Applied Biosystems, ABI PRISM 7700 Sequence Detection System User Bulletin 5). The relative efficiencies of target and reference gene amplification were assessed according to the manufacturer's instructions (Applied Biosystems, ABI PRISM 7700 Sequence Detection System User Bulletin 2).

*Immunoblotting Analysis*—Polyclonal antibodies were raised commercially by ARK Resource (Kumamoto, Japan) using purified recombinant ceST1. Mixed-staged nematodes larvae from NGM plates were collected and purified by flotation on 30% sucrose. After washing the animals with 0.1 M NaCl solution, the worm pellet was frozen at –80°C and thawed in an equal volume of extract buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 250 mM Sucrose and 1.5 mM DTT). Following sonication and centrifugation (200 × *g*, 5 min), the supernatant was transferred to a new microtube, and the same volume of extract buffer was added to the pellet for re-sonication as described above. The supernatants were then combined and centrifuged at 8,000 × *g* for 10 min at 4°C, and the supernatant was subsequently centrifuged at 100,000 × *g* for 60 min at 4°C. Protein concentrations were determined

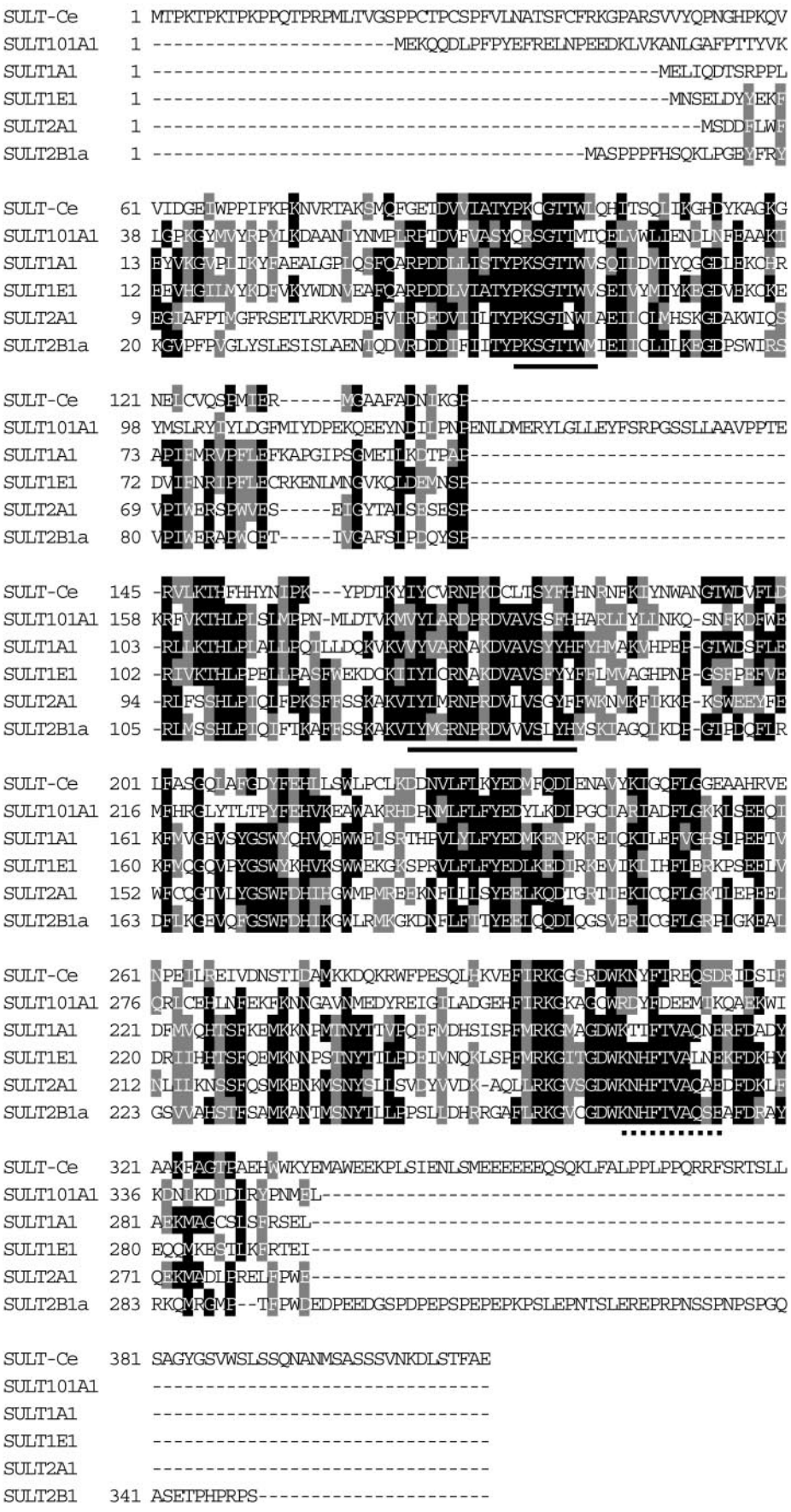


Fig. 1. **Comparison of the amino acid sequence of the *C. elegans* sulfotransferase with both human SULT isoforms and SULT101A1.** The sequences of the SULT isoforms were aligned by ClustalW and are indicated by the Boxshades. The amino acid sequence of ceST1 (PRF: 3002374J) is compared with the sequences of human SULT1A1 (PIR: JC5248), SULT1E1 (Swiss-Prot ID: SUOE_HUMAN), SULT2A1 (PIR: I38548), SULT2B1a (PRF: 2502295A) and SULT101A1 (PRF: 2218304A). The reverse text indicates an identically positioned amino acid in more than three isozymes. Shaded boxes represent amino acids that show similarities between more than three isozymes. PAPS binding sites are underlined and the dashed line indicates a KTVE motif.

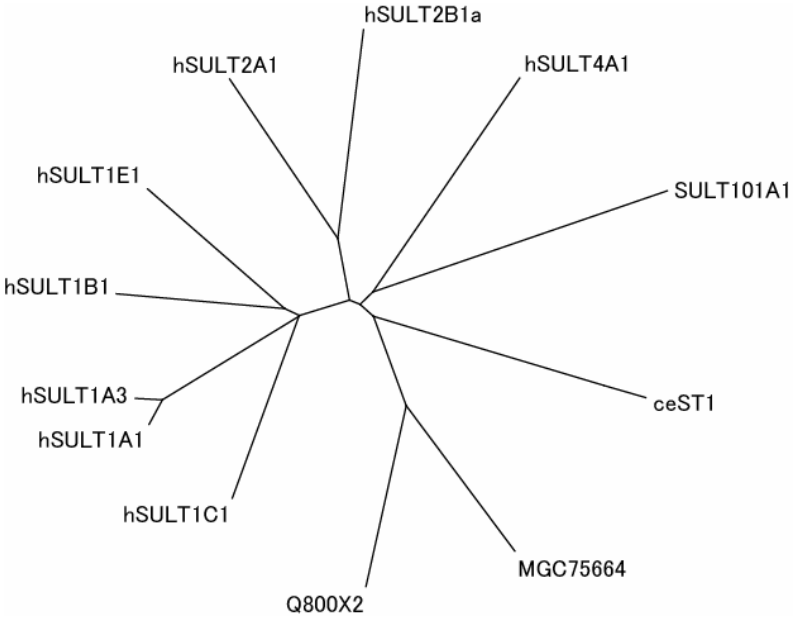


Fig. 2. **Dendrogram of the known sulfotransferases.** This dendrogram was produced by a Neighbor-Joining method using ClustalW and is based on the degree of amino acid sequence similarity between ceST1, human SULT family members and SULT homologues from *Spodoptera frugiperda*, *Xenopus tropicalis* or *Brachydanio rerio*; hSULT1A1 (PIR entry number JC5248), hSULT1A3 (Swiss-Prot ID code SUPM_HUMAN), hSULT1B1 (PIR entry number JC5885), hSULT1C1 (Swiss-Prot ID code SUC1_HUMAN), hSULT1E1 (Swiss-Prot ID number SUOE_HUMAN), hSULT2A1 (PIR entry number I38548), hSULT2B1a (PRF accession code 2502295A), hSULT4A1 (Swiss-Prot ID number S4A1_HUMAN), SULT101A1 (PRF accession code 2218304A, SULT of *Spodptera frugiperda*), MGC75664 (TrEMBL accession number Q6P353, hypothetical protein of *Xenopus tropicalis*) and Q800X2 (Protein name of UniProt Knowledgebase: Q800X2_BRARE, SULT homologue of *Brachydanio rerio*).

by the Bradford method (Bio-Rad, Hercules, CA) with bovine serum albumin standards. 0.6 μg of purified recombinant ceST1 was used as positive control, and 67 μg and 108 μg protein of cytosolic and membrane fraction, respectively, were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with rabbit anti-sera (1:2,000) and goat horseradish peroxidase—conjugated secondary antibody (1:20,000). Antibodies were visualized using the ECL plus Western Blotting Detection System.

## RESULTS

*Sequence Analysis of ceST1*—The cDNA of ceST1 was amplified by RT-PCR and cloned into pGEX6P-1. Three independent subclones were sequenced and the entire coding region of each was found to match the Y113G7A.11 region. The sequence alignment and dendrogram are shown in Figs. 1 and 2, respectively. The ceST1 cDNA was found to have an open reading frame of 1,239 bp, encoding a 412 amino acid residue protein with a calculated molecular mass of 47 kDa (Fig. 1). The deduced amino acid sequence revealed extended residues at both the amino- and carboxy-terminal ends when compared with the human SULTs (Fig. 1). In comparison with known human SULTs, the amino acid similarities with ceST1 were 30.8%, 29.9%, 30.0%, 32.4% and 31.8% for SULT1A1, 1E1, 2A1, 2B1a and 4A1, respectively. CeST1 was also found to be 29.8% identical to SULT101A1, a retinol dehydratase of *Spodoptera frugiperda*, and to contain several conserved SULT sequences, including the putative catalytic histidine residue ($His^{150}$), and $Lys^{96}$ in 5′PSB and $Ser^{177}$ in 3′PB, which have been considered to be PAPS binding sites (*12*).

*Enzymatic Characterization of ceST1*—To generate the recombinant protein, the cDNA of ceST1 was expressed in a pGEX6P-1 vector in *E. coli* BL21 cells and purified by Glutathione Sepharose 4B and Sephacryl S-200 column chromatography. The purified protein was shown to migrate at approximately 47 kDa on SDS-PAGE after removal of the GST tag with Precession Protease (Fig. 3A). Additionally, the purity of the expressed ceST1 protein in the Sephacryl S-200 fraction was measured at about 90% by Coomassie Brilliant Blue staining of the SDS-PAGE gel. Moreover, subsequent gel filtration chromatography revealed a dimer form of ceST1 with an approximate molecular mass of 88 kDa (Fig. 3B). It was confirmed with the KTVE motif, which is critical for dimerization of cytosolic sulfotransferases (*13*), was partially conserved in ceST1 (Fig. 1). In this motif, an ionic interaction between the side chains of both end amino acids is essential for dimerization. In ceST1, the corresponding amino acids were $Lys^{305}$ and $Asp^{314}$.

*Kinetic Characterization of ceST1 Activity*—The optimal pH and temperature of the enzymatic activity for processing 4-hydroxyphenylacetamide by ceST1 were determined to be 6.4–6.8 and 25°C, respectively (Fig. 4, A and B). In addition, using 150 μM 4-hydroxyphenylacetamide as the sulfate acceptor for ceST1, we calculated the $K_m$ and $k_{cat}$ value of PAPS to be 4.31 ± 0.36 μM and 0.909 ± 0.050 $s^{-1}$, respectively (Fig. 4C). The kinetic parameters of a number of xenobiotic and endogenous compounds were then measured (Table 1). This enzyme showed extensive sulfotransferase activity towards the simple phenols and bisphenol A. Furthermore, comparisons of the $k_{cat}/K_m$ values showed that bisphenol A was most efficiently sulfated by ceST1. It also showed strong activity toward 2-naphthol, 4-isopropylphenol and 4-nitrophenol. In contrast, low sulfation activity was detected for 4-nitrocatechol and 1-naphthol, and ceST1 showed no activity at all for compounds that are known to be endogenous substrates of mammalian SULTs, such as dopamine and estrone. Substrate inhibition was also observed in the reaction for bisphenol A, 2-naphthol, 4-isopropylphenol, and 4-nitrophenol (data not shown).

*Western Blotting of Endogenous ceST1*—Polyclonal antibodies were raised in rabbits against recombinant ceST1, and immunoblotting analyses were performed using both

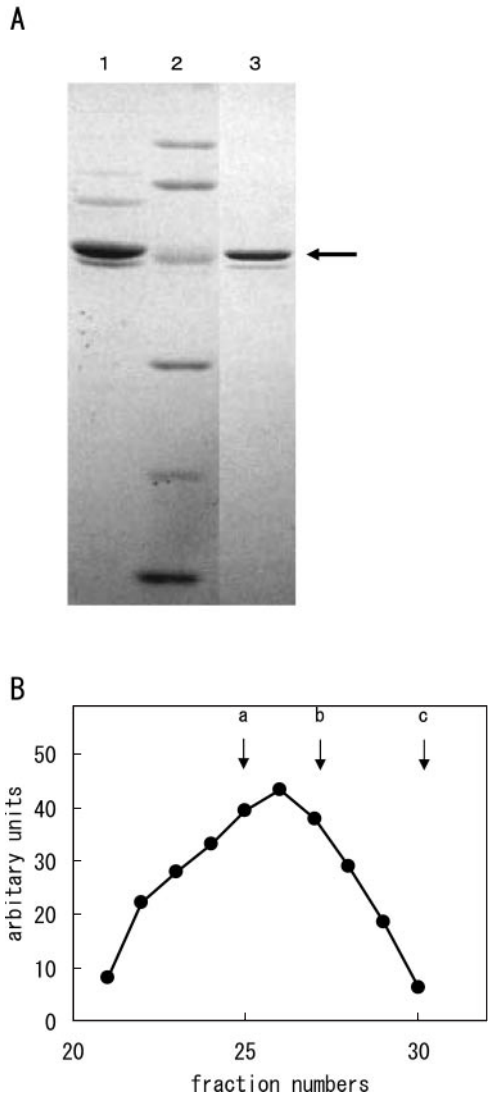


Fig. 3. **SDS—gel electrophoretic pattern of recombinant ceST1 and its elution profile from a Sephacryl S-200 gel filtration column.** (A) Purified ceST1 was subjected to SDS-PAGE on a 10% gel, followed by Coomassie Brilliant Blue staining. Lane 1, 20 μg protein of Glutathione Sepharose fraction; lane 2, molecular weight markers; lane 3, 5 μg protein of Sephacryl S-200 fraction. The protein molecular weight markers are phosphorylase *b* (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14 kDa). (B) Glutathione Sepharose fractions were applied to a Sephacryl S-200 gel filtration column. The elution profile is based on the densitometric analysis of a 47 kDa SDS-PAGE band. The peak positions of the molecular weight markers are indicated by arrows: a, phosphorylase b; b, albumin; c, ovalbumin.

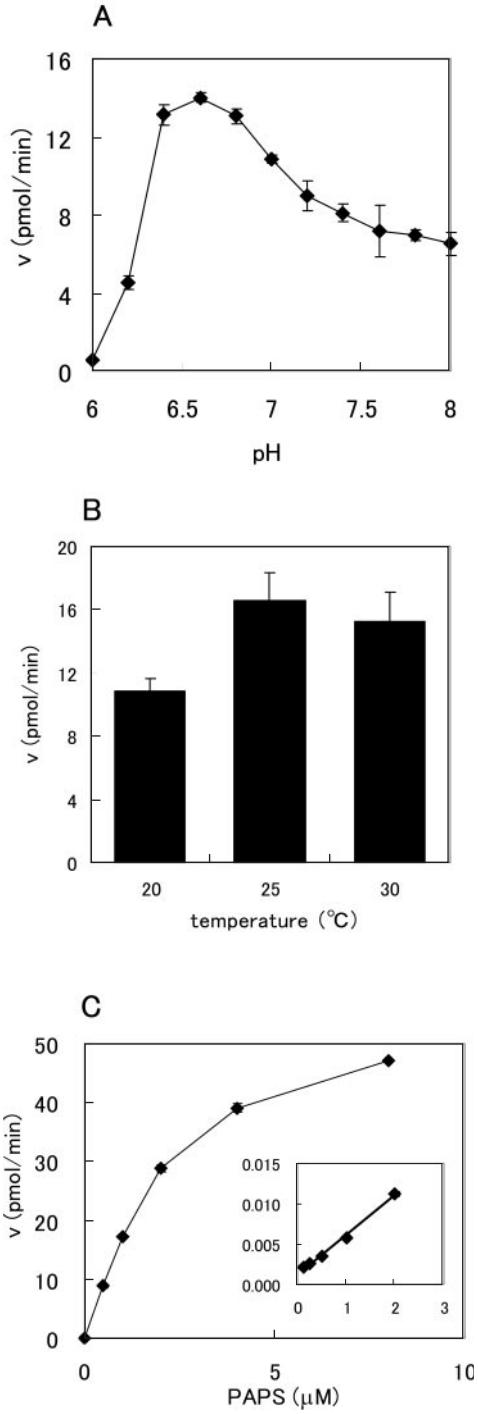


Fig. 4. **Enzymatic characteristics of ceST1.** Assays for sulfotransferase activity were carried out under conditions of various pH (A), temperature (B) or PAPS concentration (C) with 150 μM of 4-hydroxyphenylacetamide as the substrate. The experiments were carried out at 25°C in (A) and at pH 6.8 in (B) with 8 μM PAPS as the sulfate donor. (C) The Lineweaver-Burk plot is superimposed. The assays were performed in triplicate, and the results are shown as mean ± SD.

cytosolic and membrane fractions from mixtures of cultured N2 strain worms (Fig. 5). CeST1 was detected in both fractions, and both recombinant and endogenous ceST1 were found to migrate as a 47 kDa product (Fig. 5A).

*Inhibition of ceST1 by Components in the Cytosol*—Although the ceST1 protein was detectable by immunoblotting analysis of the cytosolic fraction of *C. elegans*, we could not detect sulfotransferase activity in the cytosol. To test whether this was due to the presence of inhibitors of ceST1, cytosolic fractions were prepared from N2 worms and added to assay mixtures. Sulfotransferase activity towards isopropylphenol (IPP) was markedly decreased to 33% of the control level by this addition (Fig. 5B), which was

**Table 1. Kinetic parameters of putative ceST1 acceptor substrates.**

| Substrate | $K_m$ (μM) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ ($s^{-1}\cdot M^{-1}$) |
|---|---|---|---|
| 1-naphthol (2–32 μM) | 220 ± 116 | 0.433 ± 0.233 | 1,950 ± 55 |
| 2-naphthol (1–16 μM) | 8.1 ± 0.7 | 0.533 ± 0.027 | 65,600 ± 2,100 |
| 4-nitrophenol (1–16 μM) | 11.9 ± 1.3 | 0.397 ± 0.037 | 33,500 ± 1,100 |
| 4-nitrocatechol (10–160 μM) | 43.1 ± 3.7 | 0.0198 ± 0.0016 | 460 ± 29 |
| 4-isopropylphenol | 25.3 ± 4.3 | 1.32 ± 0.12 | 52,500 ± 4,800 |
| bisphenol A | 0.55 ± 0.07 | 0.283 ± 0.025 | 513,000 ± 21,000 |
| 4-hydroxybenzoate | ND | ND | |
| Methyl 4-hydroxybenzoate | 57.6 ± 5.7 | 0.218 ± 0.014 | 3,800 ± 170 |
| 4-hydroxyphenylacetamide | 33.1 ± 3.9 | 0.461 ± 0.015 | 14,100 ± 2,200 |
| 4-aminophenol | 87.3 ± 6.1 | 0.696 ± 0.020 | 8,000 ± 770 |
| *N*-(4-hydroxyphenyl)acetamide | 530 ± 36 | 0.292 ± 0.013 | 550 ± 13 |
| 4-nitrobenzyl alcohol | 1550 ± 950 | 0.071 ± 0.038 | 49 ± 8.6 |
| dopamine (100 μM) | ND | ND | |
| tyramine (100 μM) | ND | ND | |
| octopamine (100 μM) | ND | ND | |
| synephrine (100 μM) | ND | ND | |
| tyrosine (100 μM) | ND | ND | |
| serotonin (100 μM) | ND | ND | |
| tyroxine (10 μM) | ND | ND | |
| estrone (10 μM) | ND | ND | |
| cholesterol (10 μM) | ND | ND | |
| 20-hydroxyecdysone (50 μM) | ND | ND | |

Parameters were derived from analyse of Lineweaver-Burk plots, and the results are means ± SD. ND, not detectable.

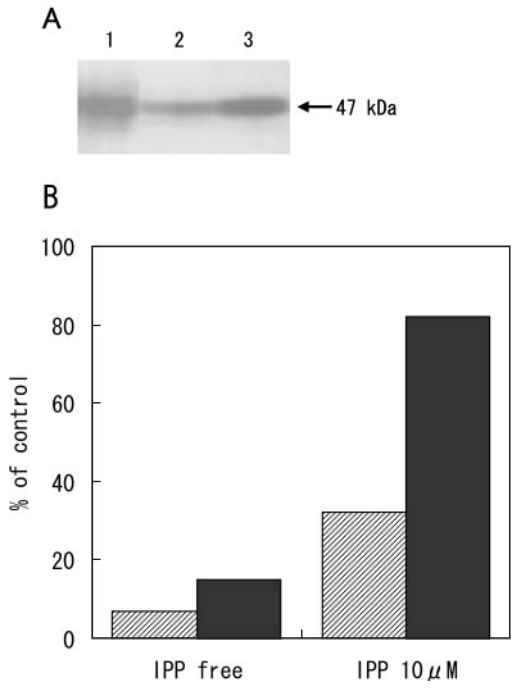


Fig. 5. **Detection of native ceST1 and its endogenous substrate.** (A) Cytosolic and membrane fractions were prepared from mixed cultured N2 worms and subjected to western blotting analysis: lane 1; 0.6 μg of recombinant ceST1, lane 2; 67 μg of cytosolic fraction, lane 3; 108 μg of membrane fraction. (B) 30 μg of protein from the cytosolic fraction was added to the assay mixture to examine its inhibitory activity and detect the presence of endogenous substrate. The closed and hatched bars indicate the residual activity with heat treated (95°C, 2min) cytosol and non-heat treated cytosol, respectively. The isopropylphenol (IPP) concentrations are indicated, and the results are the averages of duplicate determinations.

reversed by heat treatment of the extracts (95°C, 2 min). In addition, ceST1 showed low but not negligible activity in IPP-free assay mixtures (Fig. 5B), suggesting the possibility that endogenous substrates of ceST1 exist in the cytosol.

*Expression Profile of ceST1*—To examine the expression profile of ceST1 during the development of *C. elegans*, we performed real-time RT-PCR analyses using the *ama-1* gene as an internal control transcript to allow for the relative quantification of ceST1 expression. As shown in Figure 6, expression of the ceST1 gene was detected throughout development, but higher expression was evident in both the embryo and adult stages. Moreover, ceST1 transcript levels increased 29-fold in dauer larvae, which were induced by culturing *daf-2* mutants at 25°C, compared to embryos (Fig. 6B). This augmented expression was not observed when L3 larvae of the *daf-2* mutant were cultured at 25°C for 24 h (data not shown).

## DISCUSSION

In our current study, we describe the cloning and characterization of the Y113G7A.11 gene product of *C. elegans*. The gene was annotated in WormBase as a cytosolic sulfotransferase, and is, in fact, the only such SULT homologue in the *C. elegans* genome. We, therefore, tentatively designated this gene as ceST1, and subsequent amino acid sequence analysis (Fig. 1) and the generation of a phylogenic tree (Fig. 2) revealed that it belongs to a new family of SULTs. Moreover, recombinant ceST1 exists as a homodimer in bacterial lysates, similar to other known SULT enzymes (Fig. 3). Purified recombinant ceST1 also showed high sulfation activities towards simple phenol substrates under physiological conditions (Table 1), but demonstrated much higher activity towards 4-nitrophenol

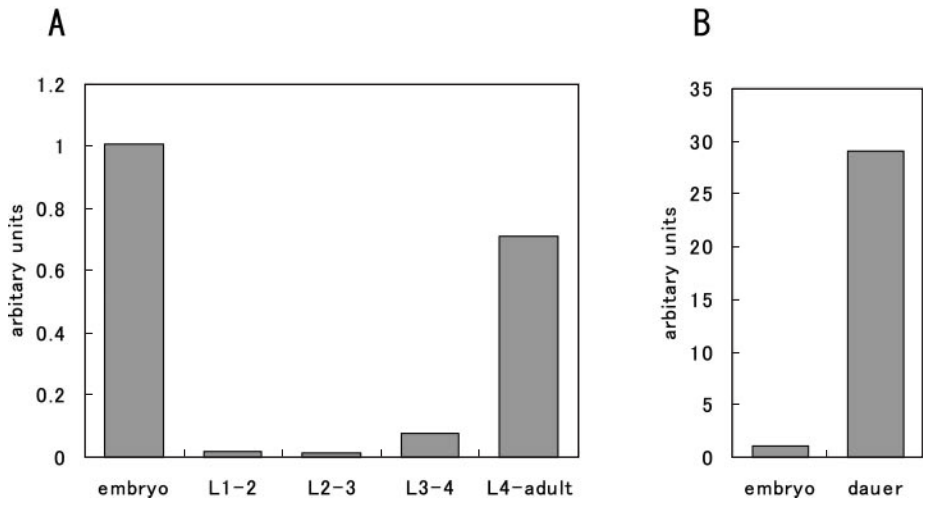


Fig. 6. **Expression profile of ceST1.** Staged larvae of the N2 strain (A) and dauer larvae of the *daf-2* (*e1370*) mutant strain (B) of *C. elegans* were collected as described in "EXPERIMENTAL PROCEDURES." Real-time RT-PCR was performed to measure ceST1 transcript levels, and ama-1 was used as the internal standard. The relative abundance was normalised to N2 strain embryos.

and 2-naphthol than towards 4-nitrocatechol or 1-naphthol. The steric exclusion of the ortho position might be responsible for this substrate preference, and, indeed, ceST1 had a higher affinity for phenolic compounds with methyl groups at the alpha carbon position. This sulfotransferase also showed low, but not negligible, sulfating activity toward cytosolic preparations of *C. elegans* (Fig. 5B), although no activity against monoamines or steroids was detectable. The highest sulfating activity of ceST1 was detected using bisphenol A as a substrate. Recently, *C. elegans* has been used in toxicity studies of different chemical substances and environmental samples, but little is known about the metabolism of xenobiotics in this organism. It was previously reported that bisphenol A increases the number of germ cells, in addition to 17β-estradiol, in *C. elegans* (*14*). Hence, the metabolism of xenoestrogens, such as bisphenol A, in *C. elegans* needs to be clarified further.

Immunoblotting analysis indicated that ceST1 exists in the cytosolic fraction of *C. elegans* (Fig. 5A). We could not detect sulfotransferase activity in the cytosol and observed heat labile inhibitory activity against ceST1 in the cytosol (Fig. 5B). We speculate that sulfatases might be responsible for this effect. The balance of sulfatase and sulfotransferase activities may be vitally important in *C. elegans* and should be further examined in future studies. We found sulfating activity of the recombinant ceST1 toward cytosolic preparations (Fig 5B). These data suggest that an unknown endogenous substrate of ceST1 may regulate germ cell proliferation as well as bisphenol A.

The dauer diapause stage of *C. elegans* is formed when environmental conditions are inadequate for successful reproduction (*15*). To induce dauer larvae, we utilised *daf-2 (e1370)* mutants, which enter the dauer stage constitutively under nonpermissive temperature conditions (*16*). *daf-2* encodes an insulin receptor family member and mutations of the FoxO ortholog *daf-16* gene rescue the phenotype caused by *daf-2* mutations (*17*). Interestingly, ceST1 was found to be expressed abundantly in the dauer larvae. Moreover, ceST1 did not seem to be directly regulated by the DAF-2 and DAF-16 signalling pathways because a temperature shift is necessary prior to the L3 stage to induce its expression (*17*). Whereas the physiological functions of ceST1 in the dauer larvae of *C. elegans* remain unclear at present, it is possible that this sulfotransferase functions either as a defence system against xenobiotics or as a regulator of germ cell proliferation.

We also detected ceST1 in both the cytosol and membrane fractions by immunoblot analysis (Fig. 5A). Although all previously known SULTs have been purified from cytosol preparations, it has been reported that human SULT2A1 is predominantly clustered around lipid droplets in the adrenal gland (*18*), and that human SULT2B1 is partly distributed to nuclei in the placenta (*19*). It is possible that the external sequence of ceST1 is a localization signal for the membrane fraction because both hSULT2B1 and ceST1 have long C-terminal extensions (Fig. 1). Further investigations about intracellular localization of ceST1 are needed.

Recently, the Y113G7A.11 gene has been designated as *ssu-1* (suppressor of stomatin uncordination) in WormBase. Mutations in stomatin disrupt a neuronal transduction mechanism in the sensory nerve cells of *C. elegans* (*20*). Further investigations of the *ssu-1* mutant phenotype will, therefore, be required to clarify the precise physiological functions of the Y113G7A.11 gene product.

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