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Dimerization Is Responsible for the Structural Stability of Human Sulfotransferase 1A1

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ABSTRACT:

Cytosolic sulfotransferases (SULTs) are responsible for the metabolism of a variety of drugs, xenobiotics, and endogenous compounds. Most cytosolic SULTs are found to be homodimers. However, transformation between monomeric and dimeric SULTs can be achieved by a single amino acid mutation. The importance of quaternary structure for cytosolic sulfotransferase was investigated using recombinant human SULT1A1, a homodimer, and its monomeric mutant (V270E). The differences between dimeric and monomeric SULT1A1 were examined by size-exclusion liquid chromatography, enzyme kinetics, substrate binding affinity, thermal inactivation, conformational stability, and circular dichroism. Variations, especially on their secondary structures and stability, between homodimer and monomer of human SULT1A1 were observed. It was found that the active site of SULT1A1 was not significantly perturbed after the change of its quaternary structure according to SULT1A1 kinetics and substrate binding affinity. However, the stability of monomeric SULT1A1 is significantly decreased. We proposed that the importance of human SULT1A1 as a homodimer was to maintain its structural stability, and the change of secondary structure was responsible for alternating its quaternary structure.

Cytosolic sulfotransferases (SULTs) are a large family of enzymes that catalyze the transfer of the sulfuryl group from the universal donor, 3′-phosphoadenosine 5′-phosphosulfate to numerous endogenous and exogenous compounds. The substrates are usually small molecules including steroid hormones, bioamines, chemical carcinogens, xenobiotics, therapeutic drugs, and neurotransmitters (Coughtrie, 2002; Strott, 2002). Some cytosolic SULTs, especially for SULT1, are phase II enzymes of detoxication that sulfonate xenobiotic substrates to become more hydrophilic and to facilitate their excretion from organisms (Glatt, 2000).

There are five SULT1 subfamilies that possess distinct, as well as overlapping, substrate specificity (Hempel et al., 2007). In humans, the relevant genetic polymorphisms of the transcribed region are known for three forms (SULT1A1, SULT1A2, and SULT1A3), which share more than 90% sequence identity (Hempel et al., 2005). SULT1A1 (accession number P50225) is mainly considered as the enzyme responsible for xenobiotic sulfonation. In the single nucleotide polymorphism database, nine allelic variants of SULT1A1 have been identified. The sequence of SULT1A1*1 is usually used to represent that of SULT1A1. The most characterized polymorphism is SULT1A1*2, which possesses a single residue substitution of histidine for arginine (R213H). This single nucleotide polymorphism has been shown to link with susceptibility to cancer, such as breast, esophageal, bladder, and colorectal cancers (Hirata et al., 2008). SULT1A1*2 is thermolabile, and is less active than SULT1A1*1 (Ozawa et al., 1999). According to the known crystal structure (Gamage et al., 2003), this mutation may affect either substrate regulation or structural stability of SULT1A1.

Most cytosolic SULTs are known as homodimers with rare exceptions. It has been shown that the dimerization interface of cytosolic sulfotransferase may locate around the substrate-binding site near the N terminus (Pedersen et al., 2000) and was proposed as the origin of substrate inhibition. However, contrary to the previous study, it was shown later that the dimerization interface is indeed the short segments of polypeptide chains near the carboxyl terminus (Petrotchenko et al., 2001). The subunits of homodimers interact through a KTVE motif, KxxxTVxxxE, which is responsible for the dimerization of cytosolic SULTs (Petrotchenko et al., 2001). It has been shown that the substrate inhibition is not related to the subunit dimerization of human SULT2A1 (Lu et al., 2008). It is reasonable to predict that mutation at this motif may convert the homodimer into monomers, and it has been shown that mutants V269E and V260E of human SULT1E1 and SULT2A1, respectively, are converted to monomers from the homodimers. Likewise, the P269T and E270V mutants of mouse SULT1E1 are converted to a homodimer from a naturally produced monomer (Petrotchenko et al., 2001). It is interesting to note that cytosolic SULTs seem to be capable of forming not only homodimers but also heterodimers. It has been reported that sulfonation of *N*-hydroxy-2-acetylaminofluorene is associated with three different dimers of SULTs from rat liver cytosol: rat SULT1A2 homodimer, rat SULT1C1 homodimer, and rat SULT1A2/SULT1C1 heterodimer

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ABBREVIATIONS: SULT, sulfotransferase; *p*NP, *p*-nitrophenol; *p*NPS, 4-nitrophenyl sulfate; PAP, 3′-phosphoadenosine 5′-phosphate; MES, 4-morpholineethanesulfonic acid; bis-tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; Vis, visible; CD, circular dichroism; PDB, Protein Data Bank.

(Kiehlbauch et al., 1995), which indicates that the KTVE dimerization motif may cross-interact within cytosolic sulfotransferase families.

A search through the database reveals that other cytosolic SULTs may be present as monomers in addition to mouse SULT1E1, such as the rat SULT1E1 (KxxxPExxxE) and *Xenopus laevis* SULT5A1 (QxxxTExxxK), which possess distinct amino acid substitutions upon characteristics within the KTVE motif. Why cytosolic SULTs exist as homodimers or monomers and the effect of quaternary structure on the catalysis and function of these enzymes have not been well studied. In this research, we explore the functional significance of the dimerization on cytosolic SULTs using human SULT1A1 as model.

Materials and Methods

Materials. *PfuTurbo* DNA polymerase and DpnI restriction enzyme were purchased from Stratagene (La Jolla, CA). 4-Nitrophenol (*p*NP), 4-nitrophenyl sulfate (*p*NPS), 3′-phosphoadenosine 5′-phosphate (PAP), β-naphthol, phenylmethylsulfonyl fluoride, urea, MES, glycine, and bis-tris propane were purchased from Sigma-Aldrich (St. Louis, MO). SDS and potassium phosphate (dibasic) were obtained from Mallinckrodt Baker (Phillipsburg, NJ). Glutathione *S*-transferase Sepharose fast flow and Sephacryl S-200 HR were obtained from GE Healthcare (Uppsala, Sweden). All other chemicals were of the highest purity commercially available.

Site-Directed Mutagenesis. The QuickChange site-directed mutagenesis kit from Stratagene was used for the generation of SULT1A1 monomeric mutant (V270E). Wild-type SULT1A1 cDNA (accession number P50225) incorporated in the pGEX-2TK expression vector was used as the template in conjunction with specific mutagenic primers. The V270E sense mutagenic oligonucleotide primer was 5′-caccttcaccgtggcgcagaatg-3′ purchased from Mission Biotech (Tapei, Taiwan). Mutated cDNA sequences were confirmed using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA) following the standard protocol.

Preparation of Wild-Type and Monomeric Mutant of SULT1A1. The expression and purification of SULT1A1 was described previously (Sakakibara et al., 1998). Both wild-type and V270E mutant of SULT1A1 were incorporated into pGEX-2TK at BamHI and EcoRI restriction sites. After treatment with thrombin, additional residues, GSRRASVGS, from vector were included on the N terminus of both mutant and wild-type SULT1A1. A homogeneous protein was obtained through GSTrap affinity columns and determined by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). The protein concentrations of the homogeneous form of wild-type and monomeric mutant of SULT1A1 were estimated on the basis of absorbance at 280 nm for 1.52 ml/mg/cm (Gill and von Hippel, 1989) using a UV/Vis spectrophotometer (UV/Vis-3300; Hitachi, Tokyo, Japan).

Size-Exclusion Chromatography. Molecular weights of the wild-type (homodimer) and V270E mutant (monomer) of SULT1A1 were estimated by size-exclusion chromatography. Aliquots of 1 ml of various samples were applied on a Sephacryl S-200 HR column (1.6 cm × 60 cm) that had been equilibrated with 20 mM Tris buffer at pH 7.5 containing 150 mM NaCl. Proteins were eluted with the same buffer at a flow rate of 1.0 ml/min. Molecular weights were estimated against calibration standards consisting of ribonuclease A (15.6 kDa), chymotrypsinogen A (19.4 kDa), ovalbumin (47.6 kDa), and albumin (62.9 kDa). The apparent molecular weights of wild-type and monomeric mutant were calculated according to the protocol of the gel filtration calibration kit.

Enzymatic Assay. To take advantage of the ease of colorimetric assays, the activities of SULT1A1 and its V270E mutant were determined according to a procedure reported previously (Yang et al., 1996; Burkart and Wong, 1999; Lin and Yang, 2000) using *p*NPS as the initial sulfuryl group donor. The reaction was monitored by the production of *p*NP, which gives strong absorption at 400 nm (ε = 10,500 at pH 7). All other compounds used in this assay including β-naphthol are transparent at this wavelength. PAP, serving as a cofactor for sulfuryl group transfer between *p*NP and β-naphthol, was first sulfated by *p*NPS to yield 3′-phosphoadenosine 5′-phosphosulfate, which in turn transferred the sulfuryl group to β-naphthol. The rate determined reflects the transfer of the sulfuryl group from *p*NPS to PAP because this reaction is monitored by the production of *p*NP. The other substrate, β-naphthol, which does not exhibit absorption at 400 nm, serves as the final sulfuryl group acceptor to facilitate the continuation of the reaction. The effective range of the SULT1A1 assay requires 0.5 to 5 μg of enzyme that gives linear responses with respected to the amount of enzyme used. The sensitivity of the colorimetric assay is much less than that of the isotope-based assay. However, it is appropriate for the assay of SULT1A1. In addition, this spectrometric assay is continuous and convenient. The reaction mixture with a final volume of 1 ml consisted of 100 mM bis-tris propane at pH 7.0, 1 mM *p*NPS, 2 μM PAP, and 50 μM β-naphthol. A sufficient amount of enzyme (0.5–5 μg) was added for detection of an absorption change at 400 nm. The reaction was carried out for 2 min in a cuvette with path lengths of 1 cm at 25°C in a Hitachi UV/Vis-3300 spectrophotometer. Under these conditions, the reaction was linear with respect to time and enzyme concentration. The data presented include calculated mean values derived from three experiments. To determine the effect of pH on enzyme activity, the following buffer systems were used: 100 mM MES at pH 6 to 7, bis-tris propane at pH 7 to 9, and glycine at pH 9 to 10. In the thermal inactivation experiment, aliquots of SULT1A1 (40 nM dimer and 80 nM monomer, respectively) were preincubated at the desired temperatures (from 25 to 50°C) for 15 min, and their activities were measured after being cooled under enzymatic assay conditions at 25°C.

Data Analysis for the Determination of Kinetic Constants. Kinetic experiments were analyzed using nonlinear regression to fit the appropriate equation to the data. Data obtained in noninhibitory experiments were individually fit to Michaelis-Menten eq. 1 (Cornish-Bowden, 1995):

$$v = \frac{V[S]}{K_m + [S]} \tag{1}$$

The apparent values of K_m and k_{cat} were determined using nonlinear regression by SigmaPlot 2001 (version 7.0; Systat Software, Inc., San Jose, CA) and Enzyme Kinetics Module (version 1.1; SPSS Inc., Chicago, IL). Data shown represent mean values derived from three determinations.

Ligand Binding. The dissociation constants (K_d) of PAP and *p*NP toward the homodimer and monomer of SULT1A1 were determined with a spectrofluorimeter as described previously (Lu et al., 2008). The decrease in intrinsic fluorescence of protein at 340 nm was observed upon excitation at 280 nm. The mixture consisted of 100 mM potassium phosphate buffer at pH 7.0 and 0.1 or 1 μM aliquot of enzyme in the individual experiment for varying concentrations of PAP or *p*NP, respectively, at 25°C with a final volume of 1.3 ml in a quartz cuvette of 1-cm square cross-section.

Urea-Dependent Unfolding of SULT1A1. In this experiment, 5 μM wild-type or monomeric mutant of SULT1A1, respectively, in 20 mM potassium phosphate buffer at pH 7.0 was treated with desired concentration of urea (0–7 M) at room temperature for 1 h as described previously (MacDonald and Pozharski, 2001). The fraction of unfolded urea-treated SULT1A1 enzyme was determined by circular dichroism at 222 nm ($CD_{222\,nm}$) using a Jasco J715 spectropolarimeter (Jasco, Tokyo, Japan) at room temperature with a cell of 1 mm pathlength. The data were analyzed by a two-state model of denaturation, in which only the native and the denatured states were populated. $CD_{222\,nm}$ (F) as a function of the urea concentration, [Urea], was given by eq. 2,

$$F = \frac{\{(\alpha_N + \beta_N[\text{Urea}]) + [(\alpha_U + \beta_U[\text{Urea}]) \times \exp[(m[\text{Urea}] - \Delta G_{UN}^{H_2O})/\text{RT}]\}}{\{1 + \exp[(m[\text{Urea}] - \Delta G_{UN}^{H_2O})/\text{RT}]\}} \tag{2}$$

where α is the intercept and β is the slope of the baseline at the native (N) or unfolding (U) state. $[\text{Urea}]_{50\%}$ is the urea concentration at the midpoint of urea denaturation, and m is a constant that is proportional to the increase in the degree of exposure of the protein on denaturation. R is the gas constant (8.314 J/mol/K), and T is the temperature in Kelvin. $\Delta G_{UN}^{H_2O}$, the free energy of denaturation in the absence of urea, is the product of $[\text{Urea}]_{50\%}$ and m, and m was obtained by nonlinear regression by fitting the data to eq. 2 using SigmaPlot 2001 (version 7.0) and Enzyme Kinetics Module (version 1.1; SPSS Inc). $[\text{Urea}]_{50\%}$ was obtained by eq. 3:

$$\Delta G_{UN}^{H_2O} = m[\text{Urea}]_{50\%} \tag{3}$$

Circular Dichroism Spectroscopy. CD measurements were performed on a Jasco J715 spectropolarimeter, and the data were processed with J-700 standard analysis version 1.33.00 (Jasco). CD spectra of enzymes were deter-

TABLE 1

Molecular masses of wild-type and monomeric mutant (V270E) of SULT1A1

SULT1A1	V_e[a]	Apparent M_r[b]	Theoretical M_r[c]
	ml	*Da*	
Wild-type	52.53	63093	68,331
V270E	62.69	37350	34,195

[a] V_e indicates the retention volume of Sephacryl S-200 HR column.
[b] The apparent molecular masses were determined according to the calibration curve by Sephacryl S-200 HR column chromatography.
[c] The theoretical molecular masses were calculated by the ProtParam on the ExPASy server (http://tw.expasy.org/tools/protparam.html).

mined at a concentration of 1 mg/ml in 20 mM phosphate buffer (pH 7.0) in a 1-cm light path length quartz cell in the near-UV range (240–320 nm) and 1-mm light path length cell in the far-UV (200–240 nm) at room temperature.

Results

Preparation of Wild-Type and Monomeric Mutant of SULT1A1. The molecular weights of wild-type and monomeric mutants of SULT1A1 were estimated by gel filtration chromatography using Sephacryl S-200 HR; the retention volume (V_e) and predicted molecular weight are shown in Table 1. The molecular weights estimated by gel filtration were similar to the theoretical molecular weights of wild-type and monomeric mutant of SULT1A1. The apparent molecular weight of wild-type SULT1A1 was approximately 2-fold that of V270E mutant. These data indicated that the interface of dimerization was easily disrupted through site-directed mutagenesis to form monomeric SULT1A1 because glutamic acid might break the hydrophobic zipper-like KTVE motif as predicted in previous studies (Petrotchenko et al., 2001).

Kinetic Analysis of Wild-Type and Monomeric Mutant of SULT1A1. Kinetic constants of wild-type and monomeric mutant of SULT1A1 are shown in Table 2 using PAP and *p*NPS as cosubstrates. The apparent K_m (PAP) and K_m (*p*NPS), respectively, between homodimeric and monomeric SULT1A1 were the same within S.E.s. The k_{cat} and k_{cat}/K_m of the dimer were approximately twice those of monomeric SULT1A1. These results indicated that each subunit of the dimer and monomer was competent catalytically toward either PAP or *p*NPS as substrate, and the catalytic mechanism might also be the same for the dimer and monomer of SULT1A1.

Dissociation Constants of PAP and *p*NP with Wild-Type and Monomeric Mutant of SULT1A1. The dissociation constants of PAP and *p*NP with dimeric and monomeric SULT1A1 are shown in Table 3. Although significant variations were observed, the dissociation constants obtained for both PAP and *p*NP between dimeric (wild-type) and monomeric SULT1A1 were on the same order of magnitude. The result indicated that the enzyme active site may remain mostly intact after the changes between the dimer and monomer of SULT1A1.

pH Profile. pH profiles of dimeric and monomeric SULT1A1 are shown in Fig. 1. The optimal pH toward wild-type and its monomeric mutant of SULT1A1 was pH 6.5. At neutral and alkaline pH values (7.0–10.0), similar specific activities were observed for both dimeric and monomeric SULT1A1. However, significant variations were observed at acidic pH values (6.0–6.5). As shown in Fig. 1, the specific activity of monomeric SULT1A1 sharply diminished at pH 6 (approximately 3% of its optimal activity at pH 6.5). In contrast, wild-type SULT1A1 remained relatively active at pH 6. The significant decrease in monomeric SULT1A1 activity at acidic pH may result from its structural instability as described later.

Thermal Stability. Thermal stability of SULT1A1 was determined by its residual specific activity after incubation at each desired temperature ranging from 25 to 50°C as shown in Fig. 2. The variation in specific activity between wide-type and monomeric SULT1A1 was strongly temperature-dependent. Temperatures at 50% thermal inactivation were approximately 42 and 50°C for monomeric and dimeric SULT1A1, respectively. The results of SULT1A1 thermal stability as shown in Fig. 2 revealed that the monomer was less stable than the homodimer and might be the cause of the loss of monomeric activities in the rigorous conditions.

Urea-Dependent Unfolding of SULT1A1. Normalized urea-induced unfolding curves of dimeric and monomeric SULT1A1 are shown in Fig. 3. $[Urea]_{50\%}$ values for urea-induced denaturation calculated (according to eqs. 2 and 3) from Fig. 3 were 4.8 and 2.5 M for wild-type and monomeric mutant of SULT1A1, respectively. This phenomenon indicated that the dimer was more structurally stable than the monomer against the urea denaturation. The denaturation curves shown in Fig. 3 also indicate that a sudden elimination of the secondary structure was observed for monomeric SULT1A1 at a urea concentration near its $[Urea]_{50\%}$.

Structural Analysis of SUL1A1 by Circular Dichroism Spectroscopy. Significant structural and conformational differences between wild-type and monomeric mutant of SULT1A1 were observed by circular dichroism spectra as shown in Fig. 4. The CD spectra in the range of the far-UV (200–240 nm) (Fig. 4A) indicated that the secondary structure was sacrificed to form monomeric SULT1A1. Prediction of the secondary structures from the CD spectra indicated that the monomer might lose a significant amount of its α-helix. In fact, the KTVE dimerization interface localizes near helix 14 (residue 270–282). Accordingly, it suggests that the single mutation (V270E) of SULT1A1 caused the change in the secondary and quaternary structures. Figure 4B shows the CD spectra of SULT1A1 in the range of the near-UV (240–320 nm). The variation at 270 to 290 nm indicated that the environments of aromatic residues, such as phenylalanine, tyrosine, and tryptophan, were different in the monomeric and dimeric SULT1A1. There were just two tryptophans (Trp-155 and Trp-264) localized near the KxxxTVxxxE motif. CD data indicated that a single mutation of V270E caused the structural change at the secondary and tertiary structures that also significantly changed the environment of hydrophobic amino acids.

Discussion

The data shown in Table 1 demonstrated clearly that a single mutation at Val-270 of human SULT1A1 created a monomer from a wild-type homodimer. Confirmed by gel filtration, human SULT1A1 was forced to become a monomer through a single mutation, and the total activity expressed was found to be similar to that of wild-type human SULT1A1. However, after the purification procedure, low recoveries for the monomeric mutant of SULT1A1 indicated that the monomer may be unstable. According to the multiple sequence alignment, the KTVE motif is highly conserved among most cytosolic SULTs. A search through the database reveals that rat SULT1E1 (KxxxPExxxE) and *X. laevis* SULT5A1 (QxxxTExxxK), which possess distinct amino acid substitutions upon characteristics within the KTVE motif, may be present as monomers. In addition, mouse SULT1E1 is found to be a naturally produced monomer and can be transformed to become a dimer by rebuilding the KTVE motif via the P269T/E270V double mutant (Petrotchenko et al., 2001). However, the human SULT1E1 is produced naturally as a dimer that maintains the KTVE motif (Petrotchenko et al., 2001). The difference between human and mouse SULT1E1 raises an interesting question as to why both monomeric and dimeric SULTs are present in different organisms and whether they are functionally comparable.

It should be noted that both wild-type and V270E mutant of

TABLE 2

Rate constants of SULT1A1 wild-type and monomeric mutant using PAP and pNPS as substrates

The reaction mixture in final volume of 1 ml consisted of 100 mM bis-tris propane at pH 7.0, 50 μM β-naphthol, and 1 mM *p*NPS plus PAP (5–1000 nM) or 2 μM PAP plus *p*NPS (0.05–20 mM), respectively. Enzyme (40 nM dimer and 80 nM monomer, respectively) was added to start the reaction. The reaction was carried out for 2 min in a cuvette with path length of 1 cm at 25°C in a Hitachi UV/Vis-3300 spectrophotometer for detection of an absorption change at 400 nm.

SULT1A1	PAP			*p*NPS		
	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m
	min^{-1}	*nM*		min^{-1}	*mM*	
Wild-type	152 ± 4	24.6 ± 3.3	6.2	402 ± 9	6.5 ± 0.3	61.8
V270E	82 ± 2	23.8 ± 2.9	3.4	199 ± 11	5.9 ± 0.8	33.7

TABLE 3

Dissociation constants of PAP and pNP in wild-type and monomeric mutant of SULT1A1

The mixture consisted of 100 mM potassium phosphate buffer at pH 7.0 and a 0.1 or 1 μM aliquot of enzyme in the individual experiment for 0 to 4 μM PAP or 0 to 100 μM *p*NP, respectively, at 25°C with a final volume of 1.3 ml in a quartz cuvette of 1-cm square cross-section.

SULT1A1	PAP	*p*NP
	nM	μ*M*
Wild-type	310 ± 14	53.9 ± 1.2
V270E	462 ± 13	41.5 ± 1.3

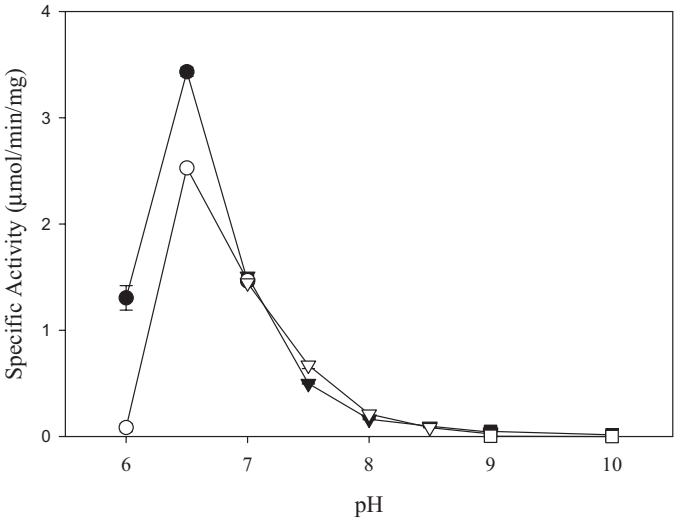

FIG. 1. pH profile of wild-type and monomeric mutant of SULT1A1. The enzymatic assays were carried out under standard assay conditions as described under *Materials and Methods*, using different buffer systems [MES at pH 6–7 (●, ○), bis-tris propane at pH 7–9 (▼, ▽), and glycine at pH 9–10 (■, □), respectively. Data shown here are the results of a typical experiment performed in triplicate. Closed and open symbols indicate the wild-type and monomeric mutant (V270E), respectively.

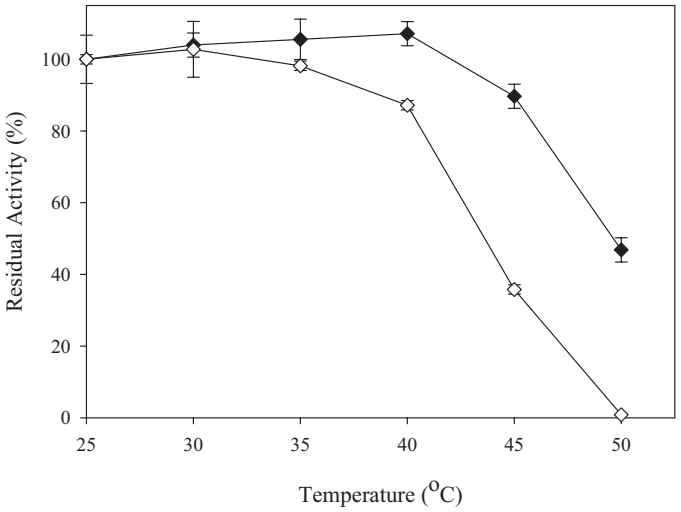

FIG. 2. Thermal stability of wild-type and monomeric mutant of SULT1A1. Specific activity of SULT1A1 was determined after the incubation at each desired temperature for 15 min over the temperature range from 25 to 50°C. The specific activities at 25°C for dimeric and monomeric SULT1A1 were 1.82 and 1.86 μmol/min/mg, respectively, and were taken as 100% activity. ◆, ◇, wild-type and V270E monomeric mutant of SULT1A1, respectively. Each point was determined from triple assay data.

SULT1A1 used in this study contained additional residues, GSRRAS-VGS, on the N terminus (Sakakibara et al., 1998). According to the crystal structure of dimeric SULT1A1 (PDB code 2D06), the dimerization motif is located apart from the N terminus at 3.43 nm (the distance between the CA atoms of N-terminal residues, Ser-8 and Val-270). It should be reasonable to assume that additional N-terminal residues had little influence on the dimerization and stability of SULT1A1. According to our results, dimerization (Table 1), stability (Figs. 2 and 3), and the secondary structure (Fig. 4) of wild-type SULT1A1 were affected mainly by a single mutation at V270E and not by this extra sequence.

Rate constants of wild-type and monomeric mutant of human SULT1A1 shown in Table 2 indicated that their catalytic properties were not significantly affected by their quaternary structures. The K_m values (for both PAP and *p*NPS) were not distinguishable between those of wild-type dimer and its monomeric mutant. The k_{cat} values (for both PAP and *p*NPS) for wild-type human SULT1A1 were twice those of its monomeric mutant, indicating that the function of each enzyme subunit was the same in either the dimeric or monomeric form. Significant variations were observed for the dissociation constants between those of wild-type and its monomeric mutant (Table 3), but the dissociation constants for PAP and *p*NP, respectively, remained on the same order of magnitude, indicating that the quaternary structure only slightly affected the binding affinity of the substrate. This observation was consistent with the rate constants shown in Table 2, and it was reasonable to propose that the active sites of dimeric or monomeric SULT1A1 were very similar.

Although the change in the quaternary structure of human SULT1A1 showed no obvious effect on the enzymatic activities, significant variations in stability were observed between the dimeric and monomeric enzyme. Monomeric SULT1A1 lost more activity than the dimeric enzyme in acidic pHs (Fig. 1). The thermal inactivation experiment indicated that the monomeric mutant was more labile than the wild-type (Fig. 2) at elevated temperature. The conformational stability examined by the urea unfolding experiment also showed that the monomer was denatured under a much lower concentration of urea (Fig. 3). The structure conformation that is responsible for the stability of a protein has been reported in many case studies (Mei et al., 2005). According to the experimental results described above, the human SULT1A1 dimer might be stabilized by

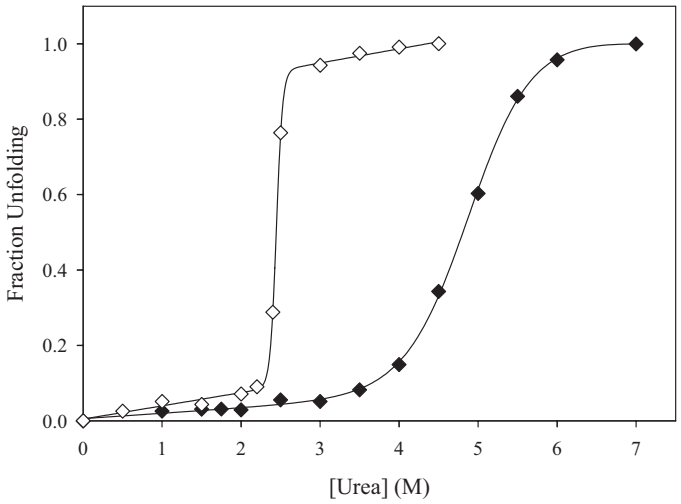

FIG. 3. Urea-induced equilibrium unfolding of conformational stability studies. Urea-induced equilibrium unfolding was monitored by ellipticity at 222 nm on circular dichroism after unfolding induction by urea at room temperature for 1 h. ♦, ◇, unfolding curves for wild-type and V270E mutant. Normalized data (fraction unfolded) were used, and the curves were obtained using nonlinear regression. Fraction unfolded values of wild-type and monomeric mutant were plotted against urea concentration with a curve (——) describing the best fit of the data to eq. 2.

protein-protein interaction that induced conformational change. This suggestion was supported by the CD spectra shown in Fig. 4 that revealed significant variations in secondary structure and conformation between the monomer and dimer of SULT1A1. Composition of the secondary structure calculated (http://www.ogic.ca/projects/k2d2/orainaldia.html) (Perez-Iratxeta and Andrade-Navarro, 2008) from Fig. 4A indicated that the α-helix structures were 63.9 and 39.9% in dimeric and monomeric SULT1A1, respectively. This observation was consistent with the known crystal structures of SULT1A1 (Gamage et al., 2003, 2005) in that the helix structure next to the KTVE motif may be denatured when it was exposed to the aqueous environment in a monomer. The calculated compositions of β-sheet structures were 3.1 and 9.5% for dimeric and monomeric human SULT1A1, respectively. The spectral change shown in Fig. 4B also suggested that the environment of some hydrophobic residues (Tyr, Phe, and Trp) had changed, most likely due to the denaturation of the partial enzyme structure when its dimeric structure was eliminated. The change in the amino acid from a hydrophobic valine to a negatively charged glutamate (V270E) may also contribute to the CD spectral variation shown in Fig. 4B. It was reasonable to suggest that the major contribution to the change in CD spectra originated from the variations in secondary and quaternary structures induced by the point mutation, V270E.

Analysis of the crystal structure of a similar sulfotransferase, hu-

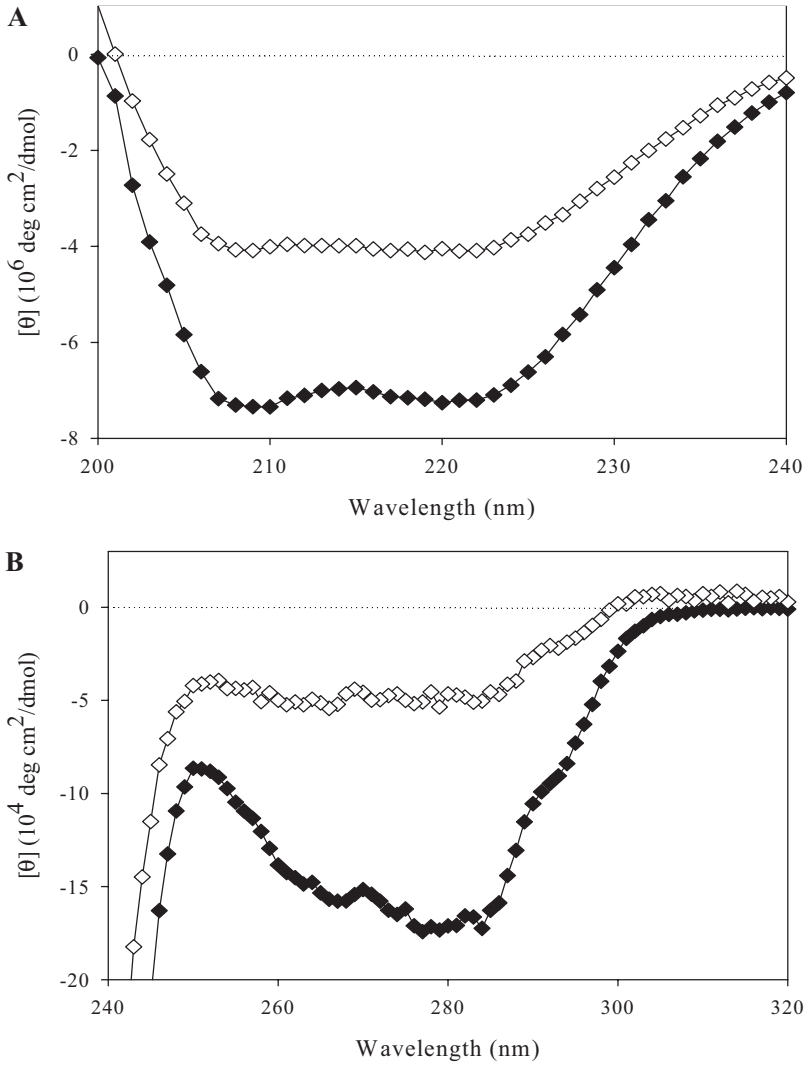

FIG. 4. Circular dichroism spectra of wild-type and monomeric mutant of SULT1A1. CD spectra of enzymes at a concentration of 1 mg/ml in the phosphate buffer at pH 7.0 were measured in (A) a 1-mm light path length quartz cell in the far-UV range (200–240 nm) and (B) a 1-cm light path in the near-UV (240–320 nm) at room temperature individually. The closed and open diamonds indicated the wild-type and monomeric mutant of SULT1A1, respectively.

man SULT1E1, also indicates that protein-protein interaction may affect its quaternary structure. V269E of human SULT1E1 is a monomer in solution (Petrotchenko et al., 2001) but is crystallized as a dimer (PDB code 1HY3) (Pedersen et al., 2002). The dimeric crystal structure of V269E mutant is similar to that of wild-type human SULT1E1 (PDB code 1G3M) (Shevtsov et al., 2003). This observation indicates that mutation at V269E does not significantly affect the structure of human SULT1E1 in a crystallized and dimeric form. However, CD spectra of SULT1A1 in the solution (Fig. 4) and its gel filtration profile (Table 1) indicated that this mutation significantly affects the secondary and quaternary structures of human SULT1A1. Thus, we speculated that the quaternary structure was important for the stability of this enzyme. However, it is also possible that mutation at V270E disrupted its nearby helix structure and affected the structural stability of SULT1A1 and then forced SULT1A1 to become a monomeric enzyme in the solution.

It has been shown that the short segments of polypeptide chains could regulate the specific protein-protein interaction and mediate important physiological processes. For example, *Saccharomyces cerevisiae* Cet1, the essential RNA triphosphatase component of the mRNA capping apparatus of budding yeast, is a homodimer (Lehman et al., 1999) and is essential for specific physiological function. Further analysis shows that the Asp-280 is required for dimer formation (Hausmann et al., 2003). Disruption of the dimer interface is lethal in vivo and renders Cet1 activity thermolabile at physiological temperatures in vitro. It has been suggested that the tunnel architecture of the active site is stabilized by the homodimeric domain and that the monomer, however, abrogates this function. In our study, the monomeric SULT1A1 became labile, but without losing its catalytic activity. The variations in secondary and tertiary structures between the dimer and monomer may be the origin of the changes in the thermal and conformational stability. We suggest that the structural variations may influence the physiological functions of the monomer and dimer of cytosolic SULTs, such as the pathways of metabolism and detoxication. It would be interesting to see whether the naturally produced monomeric SULT1E1 in mouse possesses a shorter half-life than that of dimeric SULT1E1 in human and lives transiently to regulate the estrogen sulfation. Further research is needed to uncover the significance of and difference in estrogen regulation between these two organisms.

References

Burkart MD and Wong CH (1999) A continuous assay for the spectrophotometric analysis of sulfotransferases using aryl sulfotransferase IV. *Anal Biochem* **274:**131–137.

Cornish-Bowden A (1995) *Analysis of Enzyme Kinetic Data*, pp 118–122, Oxford University Press, Oxford.

Coughtrie MW (2002) Sulfation through the looking glass—recent advances in sulfotransferase research for the curious. *Pharmacogenomics J* **2:**297–308.

Gamage NU, Duggleby RG, Barnett AC, Tresillian M, Latham CF, Liyou NE, McManus ME, and Martin JL (2003) Structure of a human carcinogen-converting enzyme, SULT1A1: structural and kinetic implications of substrate inhibition. *J Biol Chem* **278:**7655–7662.

Gamage NU, Tsvetanov S, Duggleby RG, McManus ME, and Martin JL (2005) The structure of human SULT1A1 crystallized with estradiol: an insight into active site plasticity and substrate inhibition with multi-ring substrates. *J Biol Chem* **280:**41482–41486.

Gill SC and von Hippel PH (1989) Calculation of protein extinction coefficients from amino acid sequence data. *Anal Biochem* **182:**319–326.

Glatt H (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem Biol Interact* **129:** 141–170.

Hausmann S, Pei Y, and Shuman S (2003) Homodimeric quaternary structure is required for the in vivo function and thermal stability of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* RNA triphosphatases. *J Biol Chem* **278:**30487–30496.

Hempel N, Barnett A, Gamage N, McManus ME, and Negishi M (2005) Human SULT1A sulfotransferases, in *Human Cytosolic Sulfotransferases* (Pacifici GM and Coughtrie MW eds) pp 181–232, CRC Press, London.

Hempel N, Gamage N, Martin JL, and McManus ME (2007) Human cytosolic sulfotransferase SULT1A1. *Int J Biochem Cell Biol* **39:**685–689.

Hirata H, Hinoda Y, Okayama N, Suehiro Y, Kawamoto K, Kikuno N, Rabban JT, Chen LM, and Dahiya R (2008) CYP1A1, SULT1A1, and SULT1E1 polymorphisms are risk factors for endometrial cancer susceptibility. *Cancer* **112:**1964–1973.

Kiehlbauch CC, Lam YF, and Ringer DP (1995) Homodimeric and heterodimeric aryl sulfotransferases catalyze the sulfuric acid esterification of *N*-hydroxy-2-acetylaminofluorene. *J Biol Chem* **270:**18941–18947.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:**680–685.

Lehman K, Schwer B, Ho CK, Rouzankina I, and Shuman S (1999) A conserved domain of yeast RNA triphosphatase flanking the catalytic core regulates self-association and interaction with the guanylyltransferase component of the mRNA capping apparatus. *J Biol Chem* **274:**22668–22678.

Lin ES and Yang YS (2000) Nucleotide binding and sulfation catalyzed by phenol sulfotransferase. *Biochem Biophys Res Commun* **271:**818–822.

Lu LY, Hsieh YC, Liu MY, Lin YH, Chen CJ, and Yang YS (2008) Identification and characterization of two amino acids critical for the substrate inhibition of human dehydroepiandrosterone sulfotransferase (SULT2A1). *Mol Pharmacol* **73:**660–668.

MacDonald RI and Pozharski EV (2001) Free energies of urea and of thermal unfolding show that two tandem repeats of spectrin are thermodynamically more stable than a single repeat. *Biochemistry* **40:**3974–3984.

Mei G, Di Venere A, Rosato N, and Finazzi-Agrò A (2005) The importance of being dimeric. *FEBS J* **272:**16–27.

Ozawa S, Shimizu M, Katoh T, Miyajima A, Ohno Y, Matsumoto Y, Fukuoka M, Tang YM, Lang NP, and Kadlubar FF (1999) Sulfating-activity and stability of cDNA-expressed allozymes of human phenol sulfotransferase, ST1A3*1 (^{213}Arg) and ST1A3*2 (^{213}His), both of which exist in Japanese as well as Caucasians. *J Biochem* **126:**271–277.

Pedersen LC, Petrotchenko EV, and Negishi M (2000) Crystal structure of SULT2A3, human hydroxysteroid sulfotransferase. *FEBS Lett* **475:**61–64.

Pedersen LC, Petrotchenko E, Shevtsov S, and Negishi M (2002) Crystal structure of the human estrogen sulfotransferase-PAPS complex: evidence for catalytic role of Ser^{137} in the sulfuryl transfer reaction. *J Biol Chem* **277:**17928–17932.

Perez-Iratxeta C and Andrade-Navarro MA (2008) K2D2: estimation of protein secondary structure from circular dichroism spectra. *BMC Struct Biol* **8:**25.

Petrotchenko EV, Pedersen LC, Borchers CH, Tomer KB, and Negishi M (2001) The dimerization motif of cytosolic sulfotransferases. *FEBS Lett* **490:**39–43.

Sakakibara Y, Takami Y, Nakayama T, Suiko M, and Liu MC (1998) Localization and functional analysis of the substrate specificity/catalytic domains of human M-form and P-form phenol sulfotransferases. *J Biol Chem* **273:**6242–6247.

Shevtsov S, Petrotchenko EV, Pedersen LC, and Negishi M (2003) Crystallographic analysis of a hydroxylated polychlorinated biphenyl (OH-PCB) bound to the catalytic estrogen binding site of human estrogen sulfotransferase. *Environ Health Perspect* **111:**884–888.

Strott CA (2002) Sulfonation and molecular action. *Endocr Rev* **23:**703–732.

Yang YS, Marshall AD, McPhie P, Guo WX, Xie X, Chen X, and Jakoby WB (1996) Two phenol sulfotransferase species from one cDNA: nature of the differences. *Protein Expr Purif* **8:**423–429.

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