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Mouse cytosolic sulfotransferase SULT2B1b interacts with cytoskeletal proteins via a proline/serine-rich C-terminus

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Abstract

Cytosolic sulfotransferase (SULT) SULT2B1b had previously been characterized as a cholesterol sulfotransferase. Like human SULT2B1, mouse SULT2B1b contains a unique, 31 amino acid C-terminal sequence with a proline/serine-rich region, which is not found in members of other SULT families. To gain insight into the functional relevance of this proline/serine-rich region, we constructed a truncated mouse SULT2B1b lacking the 31 C-terminal amino acids, and compared it with the wild-type enzyme. Enzymatic characterization indicated that the catalytic activity was not significantly affected by the absence of those C-terminal residues. Glutathione *S*-transferase pulldown assays showed that several proteins interacted with mouse SULT2B1b specifically through this C-terminal proline/serine-rich region. Peptide mass fingerprinting revealed that of the five SULT2B1b-binding proteins analyzed, three were cytoskeletal proteins and two were cytoskeleton-binding molecular chaperones. Furthermore, wild-type mouse SULT2B1b, but not the truncated enzyme, was associated with the cytoskeleton in experiments with a cytoskeleton-stabilizing buffer. Collectively, these results suggested that the unique, extended proline/serine-rich C-terminus of mouse SULT2B1b is important for its interaction with cytoskeletal proteins. Such an interaction may allow the enzyme to move along microfilaments such as actin filaments, and catalyze the sulfation of hydroxysteroids, such as cholesterol and pregnenolone, at specific intracellular locations.

The cytosolic sulfotransferases (SULTs) in general catalyze sulfation reactions, involving the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to a hydroxyl or an amino group of an acceptor compound [1]. Sulfation is an important, well-known pathway involved in the metabolism of not only drugs and other xenobiotics, but also endogenous compounds, including steroid and thyroid hormones, catecholamine neurotransmitters, and cholesterol in vertebrates [2–4].

On the basis of the amino acid sequences of known vertebrate SULTs, several gene families have been categorized within the SULT gene family [5–7]. Two of the major ones are the phenol sulfotransferase family (designed SULT1) and hydroxysteroid sulfotransferase family (designed SULT2) [5–7]. In humans and other mammals, the SULT2 family comprises two subfamilies, SULT2A and SULT2B, which catalyze sulfation of the 3β-hydroxyl groups of steroids with unsaturated ‘A’ rings, e.g. pregnenolone and dehydroepiandrosterone (DHEA) [8,9]. Within the SULT2B1 subfamily, two distinct members, designated SULT2B1a and SULT2B1b, have been reported to be encoded by the

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same gene, but with distinct coding mRNAs generated through alternative splicing of their exon 1, and they therefore differ only at their N-termini [9]. SULT2B1a has been characterized as a pregnenolone sulfotransferase, and SULT2B1b as a cholesterol sulfotransferase. The most remarkable feature of SULT2B1a and SULT2B1b comprises their unique and extended N-termini and C-termini, as compared with other SULTs. For SULT2B1b, the N-terminal region had been demonstrated to be essential for its catalytic activity in cholesterol sulfation [10,11], whereas the C-terminus, which contains a proline/serine-rich region, appeared to be responsible for the translocation of SULT2B1b from the cytosol into the nucleus in a tissue-specific and cell-specific manner [12,13]. Although the latter finding may have important functional implications, the physiological relevance and the underlying molecular mechanisms of nuclear translocation of SULT2B1b remain poorly understood. The orthologous *SULT2B1b* gene has been identified in mice, and mouse SULT2B1b (mSULT2B1b) was shown to contain a similar proline/serine-rich C-terminal region [14,15].

The studies reported in this article were aimed at gaining insights into the functional relevance of the proline/serine-rich region in the C-terminal tail of mSULT2B1b. Using NIH/3T3 cells stably transfected with cDNA encoding twild-type mSULT2B1b or a truncated mSULT2B1b lacking the 31 C-terminal amino acids, we obtained evidence indicating an interaction between SULT2B1b and cytoskeletal proteins.

Results and Discussion

We had previously identified, cloned and characterized *mSULT2B1b*, the protein product of which contains a unique, extended proline/serine-rich C-terminal tail (Fig. 1). mSULT2B1b, like human SULT2B1b (hSULT2B1b) [9], has since been demonstrated to be the only enzyme, among all known mouse SULTs, that is capable of catalyzing the sulfation of cholesterol [15]. As deduced from its crystal structure, the C-terminal proline/serine-rich tail of hSULT2B1b appeared to be a flexible structural element [11]. Previous studies have demonstrated that proline-rich regions of some proteins may interact with different signaling proteins, e.g. Src, PI3K and Nedd4, through their proline-dominated binding domains [16,17]. We therefore hypothesized that the proline/serine-rich C-terminal tail of mSULT2B1b may interact with other intracellular proteins, and set out to identify the proteins that it may react with.

The extended C-terminus is not involved in catalytic reaction of mSULT2B1b

Purified recombinant wild-type mSULT2B1b (mSULT2B1b-WT) or mSULT2B1b lacking the 31 C-terminal amino acids (mSULT2B1b-NT) migrated as single 38.4 kDa or 35.2 kDa bands, respectively, as calculated following SDS/PAGE (Fig. 2A). To clarify whether this extended C-terminus is involved in the catalytic activity of mSULT2B1b, enzymatic assays were performed with purified mSULT2B1b-WT and mSULT2B1b-NT, with three representative substrates, cholesterol, DHEA, and pregnenolone. Activity data indicated that mSULT2B1b-WT displayed specific activities of 45.0 ± 7.3 pmol/min/mg for cholesterol, 999.3 ± 31.7 pmol/min/mg for DHEA, and 959.9 ± 51.6 pmol/min/mg for pregnenolone. The specific activities of mSULT2B1b-NT were 45.6 ± 6.0 pmol/min/mg, 1158.5 ± 9.2 pmol/min/mg and 1106.0 ± 43.5 pmol/min/mg for cholesterol, DHEA, and pregnenolone, respectively. It therefore appears that the proline/serine-rich C-terminal tail is not required for the catalytic activity of mSULT2B1b. These activity data are comparable to those previously reported for hSULT2B1b, and are in line with the postulation that SULT2B1b catalyzes the sulfation of hydroxysteroids by its extended N-terminal guiding domain, which docks the substrate into the binding pocket of the enzyme, as supported by structural studies [10,11]. We postulated that the proline/serine-rich C-terminus may facilitate interactions with other intracellular proteins rather than executing a catalytic reaction.

mSULT2B1b interacts with cytoskeletal proteins through its proline/serine-rich C-terminal tail

The 31 amino acid C-terminal tail of mSULT2B1b contains eight prolines and eight serines (Fig. 1). Proline/serine-rich motifs have previously been shown to be important in signal transduction pathways, as well as in protein–protein interactions [16,17]. In addition to proline and serine, the C-terminal tail of mSULT2B1 contains acidic amino acids, such as aspartic acid and glutamic acid. To further investigate the functional relevance of the C-terminal tail of mSULT2B1b, protein–protein interactions were analyzed with glutathione *S*-transferase (GST) pulldown assays. Stable mSULT2B1b transfectants, GST–mSULT2B1b-3T3, GST–mSULT2B1b-NT-3T3, and GST–TAIL-3T3, were generated with the use of mouse NIH/3T3 cells, which express no detectable SULT2B1b, and the expression of corresponding recombinant proteins was verified by immunoblotting (data not shown). Figure 2B shows the proteins that bound specifically to the proline/serine-rich C-terminal tail of mSULT2B1b. It appeared that the electrophoretic patterns of the proteins pulled down by GST–WT and GST–TAIL were quite similar. Five specific protein bands detected in the GST–TAIL-3T3 fraction were excised from the gel and analyzed by peptide mass fingerprinting (PMF), using MALDI-TOF MS. The results shown in Table 1 revealed that, of the five proteins, three were cytoskeletal proteins (actin, α-actinin, and myosin) and two were molecular chaperones (HSP90 and HSC70). Many of the molecular chaperones are known to interact with cytoskeletal elements such as microfilaments and intermediate filaments, and regulate the folding of cytoskeletal or cytoskeleton-related proteins [18]. Members of the HSP70 family, including HSC70, are known to induce actin polymerization and stabilize actin filaments, and α-actinin causes organization of actin filament bundles by attaching between these filaments [19,20]. The results from the GST pulldown assay therefore indicated that mSULT2B1b may interact with actin filaments through its proline/serine-rich C-terminal tail, and additional proteins, such as α-actinin, HSP90, and HSC70, were also pulled down because of their interaction with the actin filaments. In view of this latter finding on the SULT2B1b–cytoskeletal protein complexes, it is possible that other protein bands shown in Fig. 2B may also contain actin filament components. Indeed, additional analyses revealed that three of them were, respectively, actin-related protein 2 (SwissProt accession no. P61161), myosin-9 (Q8VDD5), and myosin regulatory light chain 2-B (Q3THE2) (data not shown). It should be pointed out, however, that the exact structures in these proteins that interact with the proline/serine-rich C-terminal tail of mSULT2B1b, i.e. the ligands of the C-terminal tail of mSULT2B1b, remain to be clarified. Previous studies have shown that profilin, which regulates the dynamics of actin polymerization, acts by mediating the interaction between proline-rich proteins and actin as hubs, thereby contributing to cell migration and cell capillary morphogenesis [21,22]. Profilin, however, binds the poly(L-proline) stretches, which consist of a consensus sequence G/LPPPPPP, and would therefore probably not bind SULT2B1b [17,21]. It should also be noted that the C-terminal amino acid sequence of SULT2B1b is different from those of the SH3 and WW domains, whose major consensus motifs are PxxP and PPxY, where x denotes any amino acid [17]. The C-terminal amino acid sequence of mSULT2B1b contains the regulatory spaced proline residues (PDPEPSPSP). Previous studies have demonstrated that the WW domain of peptidyl-prolyl *cis/trans* isomerase, Pin1, interacts specifically with proteins that are phosphorylated at their S/T-P motifs [17]. We have attempted to examine whether the serine residues of the C-terminal tail of mSULT2B1b may be subjected to phosphorylation. However, no phosphorylation of the C-terminal tail of mSULT2B1b was detected (data not shown). Further investigation will be needed in order to clarify in detail the structural determinants of the interaction between the proline/serine-rich C-terminal tail of mSULT2B1b and its ligands.

mSULT2B1b is associated with the cytoskeleton through its proline/serine-rich C-terminal tail

To gain additional evidence for the interaction between mSULT2B1b and the cytoskeleton, cosedimentation experiments with a cytoskeleton-stabilizing buffer (CSB) were performed. This is a commonly employed *in vitro* biochemical method used to isolate cytoskeletal protein fractions [23]. The isolated cytoskeletal fractions were analyzed by immunoblotting with a polyclonal antibody against mSULT2B1, which showed that GST–WT was more abundant in the sedimented cytoskeletal fraction than GST–NT (Fig. 3). The trace amount of GST–NT cosedimented might have been attributable to nonspecific contamination of the sedimented cytoskeletal fraction. Nevertheless, the results provided additional evidence indicating the association of mSULT2B1b with the cytoskeletal protein fraction through its proline/serine-rich C-terminal tail.

Concluding remarks

In this study, we demonstrated that mSULT2B1b interacts with intracellular proteins, particularly cytoskeletal proteins. We postulate that mSULT2B1b may catalyze sulfation of hydroxysteroids, including cholesterol and pregnenolone, at specific intracellular locations. This may be achieved as mSULT2B1b moves along microfilaments such as actin filaments by using its unique proline/serine-rich C-terminal tail as an attachment point, similar to the mechanism employed by myosin motors. Myosin motors have been shown to move towards the barbed (+) or pointed (−) ends of actin filaments [24,25]. It should be noted that, whereas mouse, rat and human SULT2B1 enzymes all contain a proline/serine-rich C-terminal tail, there is some variation in the exact amino acid sequence in this region among the three enzymes [26]. It is therefore likely that the motif that is important for the binding of SULT2B enzymes to microfilaments, although not yet elucidated, is likely to be short in length. In spite of this unresolved issue, it is possible that the nuclear translocation of SULT2B may occur by its movement along microfilaments as mediated by its C-terminal tail. Further studies are warranted in order to fully clarify this important issue.

Experimental procedures

Materials

NIH/3T3 mouse embryonic fibroblasts (TKG0299) were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The pBluescript II SK (+) cloning vector, and *Escherichia coli* host strains XL1-Blue MRF′ and BL21, were from Stratagene (La Jolla, CA, USA). Isopropyl thio-β-D-galactoside was purchased from Takara (Osaka, Japan). The pGEX-4T-2 prokaryotic GST-fusion expression vector, glutathione–Sepharose 4B and ECL Plus reagents were from GE Healthcare (Little Chalfont, UK). The mammalian expression vector pEF6/V5-His C, Lipofectamine, Lipofectamine Plus reagent and OPTI-MEM were purchased from Invitrogen (Carlsbad, CA, USA). Oligonucleotide primers and the Ligation-Convenience Kit were products of NIPPON EGT (Toyama, Japan). KOD-plus polymerase was from Toyobo (Osaka, Japan). Blasticidin S HCl was obtained from Merck Calbiochem (Darmstadt, Germany). Protease inhibitor cocktail tablets, EDTA-free, were purchased from Roche Diagnostics (Basel, Switzerland). Cholesterol, DHEA, pregnenolone, a monoclonal antibody against β-actin (clone AC-15) and DMEM were obtained from Sigma-Aldrich (St Louis, MO, USA). MS Grade Trypsin Gold (Catalog no. V5280) was purchased from Promega (Madison, WI, USA). Anti-rabbit IgG and anti-mouse IgG, horseradish peroxidase-conjugated (Catalog nos. 7074 and 7076), were from Cell Signalling Techonology (Danvers, MA, USA). Polyclonal antibody against mouse SULT2B1 was raised in rabbit, and the antibodies therein were affinity-purified using purified recombinant mSULT2B1 covalently bound to Affi-Gel 10 Gel (Bio-Rad Laboratories, Hercules, CA,

USA), according to the manufacturer's instructions. Purified antibodies were stored in 50% glycerol solution at 0.2 mg/mL. All other chemicals were of the highest grade commercially available.

Preparation of vector constructs harboring full-length or truncated mSULT2B1

A cDNA encoding the full-length mSULT2B1b (encompassing all 338 amino acids; designated mSULT2B1b-WT) was generated by PCR amplification using an expressed sequence tag cDNA clone (Clone ID 445155) as a template, in conjunction with gene-specific sense (5′-GGCGAATTCCCATGGACGGGCCGCAGCCCC-3′) and antisense (5′-GGCGAATTCTTATTGTGAGGATCCTGGGTT-3′) oligonucleotide primers, designed on the basis of the nucleotide sequence of *mSULT2B1b* (NCBI GenBank accession no. AF026072), with *Eco*RI sites incorporated at the 5′-end and 3′-end. Amplification conditions were 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C. The mSULT2B1b cDNA thus amplified was first cloned into pBluescript II SK (+), and then subcloned into the *Eco*RI site of the pGEX-4T-2 prokaryotic expression vector. To generate the cDNA encoding mSULT2B1b that lacks the C-terminal tail (spanning amino acids 1–307, designated mSULT2B1b-NT), pBluescript harboring the full-length mSULT2B1b cDNA was used as a template for PCR amplification with specific sense (5′-GGCGAATTCCCATGGACGGGCCGCAGCCCC-3′) and antisense (5′-CCGGAATTCTTAGTCCCAGGGGAACCTCT-3′) oligonucleotide primers. The amplified cDNA was subcloned into the *Eco*RI site of pGEX-4T-2. For GST pulldown assays, to prepare the GST-fusion mammalian expression vector (pEF6-GST), pGEX-4T-2 was used as a template for GST cDNA amplification on the basis of PCR with specific sense (5′-CGGGATCCATGTCCCCTATACTAGGTTAT-3′) and antisense (5′-GGGTCATGGCTGCGCCCCACA-3′) primers. The amplified cDNA was subcloned into the *Bam*HI site of the pEF6/V5-His C mammalian expression vector. To generate the cDNA encoding the C-terminal tail of mSULT2B1b (31 amino acids spanning amino acids 308–338; designated TAIL) that contains the proline/serine-rich region, specific sense (5′-CCGGAATTCCACGTCTGAAGAGGATAGC-3′) and antisense (5′-GGCGAATTCTTATTGTGAGGATCCTGGGTT-3′) primers were used. The PCR-amplified cDNA was subcloned into the *Eco*RI site of pEF6–GST. pEF6–GST–mSULT2B1b-WT and pEF6–GST–mSULT2B1b-NT were similarly generated by ligating *Eco*RI-restricted mSULT2B1b-WT or mSULT2B1b-NT PCR product into the *Eco*RI-restricted pEF6–GST mammalian expression vector.

Stable expression of mSULT2B1b in NIH/3T3 cells

NIH/3T3 mouse embryonic fibroblasts were routinely maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C and 5% CO_2. NIH/3T3 cells, grown to 80% confluence in 100 mm culture dishes, were individually transfected with pEF6 plasmids (GST–mSULT2B1b-3T3, GST–mSULT2B1b-NT-3T3, and GST–TAIL-3T3), by Lipofectamine and Lipofectamine Plus reagents, using standard procedures. The transfected cells were maintained in the above-mentioned culture medium for 48 h. After a 48 h incubation, the cells were passaged in the same culture medium supplemented with 10 μg/mL Blasticidin S HCl, until distinct colonies appeared. Subsequently, cells derived from each colony clone were analyzed for the expression of the expected recombinant proteins.

Bacterial expression and purification of the recombinant mouse SULTs

pGEX-4T-2 harboring either the full-length mSULT2B1b-WT or mSULT2B1b-NT was transformed into competent *E. coli* BL21 cells. Transformed BL21 cells were grown to a $D_{600\ nm}$ of ~ 0.2 in LB medium supplemented with 100 μg/mL ampicillin, and induced with 0.1 mM isopropyl thio-β-D-galactoside for 4 h. The recombinant mouse SULTs were purified

on the basis of a previously developed procedure [27]. Briefly, the collected cells were homogenized with an Ohtake French Press, recovered by centrifugation, and purified by affinity chromatography using glutathione–Sepharose; this was followed by thrombin digestion to release the recombinant protein. Protein concentration was determined according to Lowry's method, with BSA as the standard [28]. SDS/PAGE was performed on 12% polyacrylamide gels, using Laemmli's method [29].

Enzymatic assay

Sulfation activity was assayed using [^{35}S]PAPS (45 Ci/mmol) as the sulfate donor; this was synthesized from ATP and [^{35}S]sulfate by using recombinant human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase, as described previously [30]. The assay mixture, with a final volume of 25 μL, contained 50 mM Hepes/NaOH (pH 7.5), 0.4 μM [^{35}S]PAPS, and 10 μM substrate: pregnenolone, DHEA, or cholesterol. The reaction was initiated by the addition of the enzyme, allowed to proceed for 30 min at 37 °C, and terminated by heating at 100 °C for 3 min. The precipitates formed were removed by centrifugation, and the supernatant was analyzed for ^{35}S-labeled sulfated products by using a silica gel TLC procedure, with ethyl acetate/n-butanol (2 : 1; v/v) as the solvent system. The silica gel plates were then air-dried, and analyzed with an FLA-3000G fluorescent image analyzer (Tokyo, Japan).

GST pulldown assay and protein identification by PMF analysis

To assess protein–protein interactions between mSULT2B1b and intracellular proteins, stably transfected cells, grown to confluence in 10 150 mm culture dishes, were scraped off and lysed in 1 mL of lysis buffer [50 mM Hepes/NaOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 50 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride, protease inhibitor cocktail] for 30 min at 4 °C. The cell lysate was subjected to centrifugation twice at 20 400 ***g*** for 20 min at 4 °C, and the supernatant collected was fractionated with glutathione–Sepharose for 30 min at 4 °C. The Sepharose beads were then spun down and washed four times with a radioimmunoprecipitation assay buffer (50 mM Tris/HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate), suspended in SDS sample buffer, heated at 98 °C for 3 min, and resolved by SDS/PAGE. For in-gel digestion, the protein bands visualized by silver staining were excised from the gel slab, and dehydrated in 100% acetonitrile. The dehydrated gel pieces were reduced in 10 mM dithiothreitol/25 mM NH_4HCO_3 solution for 1 h at 55 °C, and subsequently alkylated in 55 mM iodoacetamide/25 mM NH_4HCO_3 solution for 45 min at room temperature. The gel pieces were then washed in 25 mM NH_4HCO_3, dehydrated again, and finally trypsin-digested in 10 ng/μL Trypsin Gold/50 mM NH_4HCO_3, with 0.1% n-octyl-β-D-glucoside solution, for 12 h at 37 °C. Trypsinized peptides were then extracted into 5% trifluoroacetic acid/50% acetonitrile solution. For PMF analysis, eluted peptides were applied on a MALDI sample plate that was covered with the matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in acetone). Mass spectra were obtained with an autoFLEX II TOF/TOF (Bruker Daltonics, Billerica, MA, USA), and the data were analyzed by a MASCOT search against the SwissProt database.

Cosedimentation analysis with CSB and immunoblot analysis

Stably transfected cells, grown to confluence in a 150 mm culture dish, were scraped off, and lysed in 200 μL of CSB (5 mM Hepes/KOH, pH 7.5, 2 mM EGTA, 1% Triton X-100, 1 mM phenylmethanesulfonyl fluoride) for 30 min at 4 °C. The homogenate was centrifuged at 200 ***g*** for 10 min at 4 °C to remove the crude nuclear fraction, and the supernatant was subjected to centrifugation at 20 000 ***g*** for 20 min at 4 °C to sediment the cytoskeletal fraction. The pellet was washed twice in CSB, suspended in SDS sample buffer, heated at 98 °C for 3 min, resolved by SDS/PAGE, and electroblotted onto an Immobilon-P membrane

[31]. The membrane was blocked with 5% nonfat milk in NaCl/P_i with 0.1% Tween-20 for 1 h, probed with rabbit polyclonal antibody against mSULT2B1 at a dilution of 1 : 200 overnight at 4 °C or mouse monoclonal antibody against β-actin at a dilution of 1 : 5000 for 1 h, washed with NaCl/P_i containing 0.1% Tween-20, and incubated with anti-rabbit IgG (for SULT2B1b) or anti-mouse IgG (for β-actin), horseradish peroxidase-conjugated, at a dilution of 1 : 1000 for 1 h. The immunoreactive bands were visualized with the ECL Plus detection system, according to the manufacturer's instructions.

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Abbreviations

CSB	cytoskeleton-stabilizing buffer
DHEA	dehydroepiandrosterone
GST	glutathione *S*-transferase
hSULT2B1b	human cytosolic sulfotransferase 2B1b
mSULT2B1b	mouse cytosolic sulfotransferase 2B1b
mSULT2B1b-NT	mouse cytosolic sulfotransferase 2B1b lacking the 31 C-terminal amino acids
mSULT2B1b-WT	wild-type mouse cytosolic sulfotransferase 2B1b
PAPS	3′-phosphoadenosine 5′-phosphosulfate
PMF	peptide mass fingerprinting
SULT	cytosolic sulfotransferase

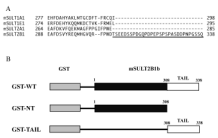

Fig. 1.

The unique extended C-terminal amino acid sequence of mSULT2B1. (A) Amino acid sequence alignment of the C-terminus of mouse SULT1A1 (SwissProt accession no. P52840), SULT1E1 (P49891), SULT2A1 (P52843), and SULT2B1 (O35400). Sequence alignments were performed with the CLUSTAL W algorithm [32]. The 31 C-terminal amino acids are underlined. (B) Construction of GST-fusion mammalian expression vectors used in the generation of stably transfected NIH/3T3 cells. pEF6/V5-His C was used as the mammalian expression vector. WT, wild-type mSULT2B1b; NT, mutant mSULT2B1b lacking the 31 C-terminal amino acids; TAIL, the 31-residue C-terminal sequence.

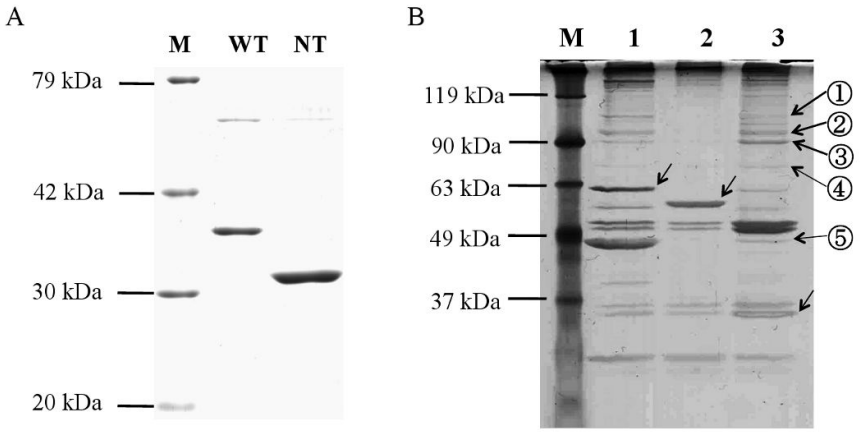

Fig. 2.

Function of the extended C-terminus of mSULT2B1b. (A) Purified recombinant enzymes, prepared as described in Experimental procedures, were resolved by SDS/PAGE on a 12% gel, and this was followed by Coomassie Blue staining. WT refers to wild-type mSULT2B1b, and NT to mutant mSULT2B1b lacking the 31 C-terminal amino acids. Coelectrophoresed protein molecular mass markers were trypsin inhibitor (20 000), carbonic anhydrase II (30 000), aldolase (42 000), and BSA (79 000). (B) Analysis of the interaction between mSULT2B1b and intracellular proteins by GST pulldown assay. The figure shows the proteins presents in transfectant cell lysates that were pulled down by glutathione–Sepharose 4B beads: lane 1, GST–mSULT2B1b-3T3; lane 2, GST–mSULT2B1b-NT-3T3; lane 3, GST–TAIL-3T3. The samples were resolved by SDS/PAGE on a 12% gel, followed by silver staining. The arrowheads indicate the GST-fusion proteins corresponding to GST–WT, GST–NT, and GST–TAIL, respectively. The protein band numbers on the right correspond to the numbers assigned for identified proteins in Table 1. Coelectrophoresed protein molecular mass markers are carbonic anhydrase (37 000), ovalbumin (49 000), glutamate dehydrogenase (63 000), BSA (90 000), and β-galactosidase (119 000).

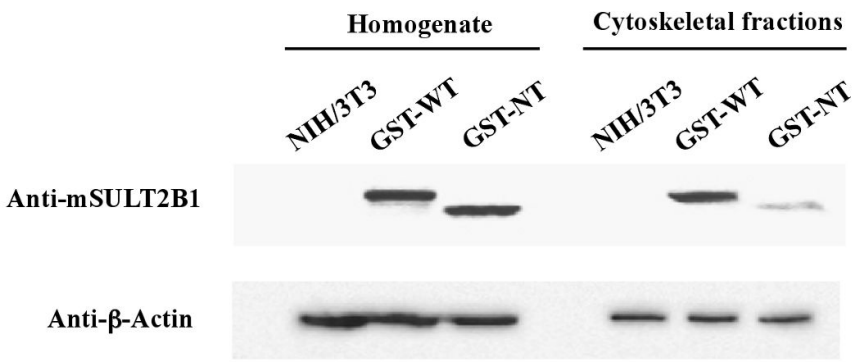

Fig. 3.

Localization of mSULT2B1b to the cytoskeletal fraction. Immunoblot analysis of the cytoskeleton fractions and crude homogenates of NIH/3T3 cells, GST–WT-3T3 cells, and GST–NT-3T3 cells was performed using rabbit polyclonal antibody against mouse SULT2B1b (upper column) or monoclonal antibody against mouse β-actin (lower column). The cytoskeletal fractions were generated with the method employing CSB, as described in Experimental procedures.

Table 1

Identification of proteins binding specifically to mSULT2B1b through its proline/serine-rich C-terminal tail. Protein bands are numbered according to Fig. 2.

Number	Identified proteins	SwissProt accession no.	Calculated molecular mass (kDa)
1	Myosin-Ic	Q9WTI7	118.9
2	α-Actinin-1 and/or α-actinin-4	Q7TPR4/P57780	103.6/105.4
3	HSP90-β	P11499	83.3
4	HSC 70	P63017	71.1
5	β-Actin and/or γ-actin	P60710/P63260	42.1/42.1