Short Communication

Subcellular Location and Molecular Mobility of Human Cytosolic Sulftotransferase 1C1 in Living Human Embryonic Kidney 293 Cells

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ABSTRACT:

Cytosolic sulfotransferases were first isolated from the hepatic cytosol, and they have been localized in the cytoplasm of formaldehyde-fixed cell samples. The current work was carried out to determine the subcellular localization and molecular mobility of cytosolic sulfotransferases in living human embryonic kidney (HEK) 293 cells. In this work, the subcellular location of human cytosolic sulfotransferase 1C1 (SULT1C1) was studied in cultured HEK293 cells using confocal laser-scanning microscopy. A green fluorescent protein (GFP)-tagged SULT1C1 protein was localized in the cytoplasm of living HEK293 cells. This is consistent with results from previous studies on several other cytosolic sulfotransferase isoforms. Fluorescence recovery after photobleaching microscopy was performed to assess the molecular mobility of the expressed GFP-SULT1C1 molecules. The results suggested that the expressed recombinant GFP-SULT1C1 molecules in living HEK293 cells may include both mobile and immobile populations. To obtain additional insights into the subcellular location of SULT1C1, two machine learning algorithms, Sequential Minimal Optimization and Multilayer Perceptron, were used to compute the probability distribution for the localization of SULT1C1 in nine selected cellular compartments. The resulting probability distribution suggested that the most likely subcellular location of SULT1C1 is the cytosol.

Introduction

Sulfoconjugation, catalyzed by cytosolic sulfotransferases, is an important biotransformation reaction for a number of endogenous and xenobiotic substances including drugs and environmental pollutants (Jakoby and Ziegler, 1990; Duffel, 1997; Falany, 1997; Weißelboum et al., 1997; Nagata and Yamazoe, 2000). As a result, the cytosolic sulfotransferase superfamily has attracted considerable attention in pharmacological and toxicological research. Studies on the subcellular location and molecular mobility of cytosolic sulfotransferases in living cells could provide additional insights into the roles of these enzymes in human cell biology and drug and xenobiotic metabolism. Mammalian cytosolic sulfotransferases were first isolated from cytosolic samples (Jakoby and Ziegler, 1990) and have been localized in the cytoplasm of mammalian cells by immunohistochemical and fluorescence protein tagging methods (Whitnall et al., 1993; Wang et al., 2004; Kapoor et al., 2007; Salman et al., 2009). The presence of mammalian cytosolic sulfotransferases in the cytoplasm of formaldehyde-fixed human cell samples has been consistent among previous studies, suggesting that the primary endogenous substrates of cytosolic sulfotransferases may exist in the cytoplasm (Whitnall et al., 1993; Wang et al., 2004; Kapoor et al., 2007). Mammalian cytosolic sulfotransferases might also associate with certain subcellular compartments such as the nucleus and endoplasmic reticulum (Whitnall et al., 1993).

Studies on protein molecular mobility are important because they may generate useful hints on the protein’s interactions with other intracellular macromolecules or membrane systems within living cells (Day, 2005). Such information could provide new insights into the molecular mechanism for the pharmacological roles of the protein. The mobility rate of an intracellular protein in living cells, which depends on the protein’s molecular size, physiochemical properties, membrane association, and interactions with other intracellular macromolecules and organelles, can be determined by using the fluorescence recovery after photobleaching (FRAP) assay (Day, 2005). Protein molecules dissolved in the intracellular fluid tend to be mobile, whereas molecules attached to intracellular membrane systems or other macromolecules tend to be immobile.

Human cytosolic sulfotransferase 1C1 (SULT1C1) (EMBL accession number AB008164) was used as a model enzyme in this work to explore the subcellular location and molecular mobility of human cytosolic sulfotransferases in living cells. Human SULT1C1 is a member of the cytosolic sulfotransferase 1C subfamily, and the reported substrates of the SULTC subfamily include several toxicologically important hydroxyl and N-hydroxyl carcinogenic chemicals such as N-hydroxy-2-acetylaminofluorene (Heflich and Nef, 1994). The SULT1C enzymes may also contribute to human thyroid hormone metabolism (Li et al., 2000). SULT1C1 was tagged with green fluorescent protein isoforms.
Materials and Methods

Cell Culture. The human HEK293 cell line was purchased from Invitrogen (Carlsbad, CA) and routinely cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum and 0.2 mg/ml G418. GFP-SULT1C1 in living HEK293 cells was detected and imaged with confocal laser-scanning microscopy. The fluorescent signal of the GFP-tagged SULT1C1 protein in a defined area of a transfected HEK293 cell body was photo-bleached by an argon (488 nm) laser (Olympus) with maximal power for 4 min. The values for laser power and time of exposure were set empirically and able to reduce the fluorescent signal of GFP-SULT1C1 by 70% (Figs. 2 and 3). The photo-bleached area was monitored at 510 nm with confocal laser-scanning microscopy at different time intervals for 10 min, and the images collected were analyzed with the Fluoview software program (Olympus).

Prediction of Protein Subcellular Location by Sequential Minimal Optimization and Multilayer Perceptron. Two machine learning algorithms, sequential minimal optimization (SMO) and multilayer perceptron (MLP) (Vapnik, 1997; Gardner and Dorling, 1998; Platt, 1998), implemented in the Weka workbench (Witten and Frank, 2002), were used for prediction of the SULT1C1 subcellular location. A summary of the specific settings for SMO and MLP (Supplemental Table 1) and the results of training set evaluation and 10-fold cross validation (Supplemental Table 2) have been provided. The training and testing sets consisted of the physicochemical profiles of 4551 previously annotated human protein sequences obtained from release 45.0 of the SWISS-PROT database (Bairoch and Apweiler, 2000). The designated subcellular localizations of the 4551 proteins were based on their associated annotations within the SWISS-PROT database. Only instances with experimentally derived annotations were included. Protein sequences with uncertain annotations and those known to shuttle between multiple subcellular compartments were not included. For each protein sequence, a set of 125 structural attributes relevant to the physicochemical properties of component amino acid side chains were computed using Lisp code. These 125 attributes captured the composition, transitions, and distributions of the component amino acids in the light of selected structural and physicochemical properties as described previously (Acquaah-Mensah et al., 2006). The resulting structural and physicochemical profiles generated for the set of 4551 human proteins were then used for machine training.

Results and Discussion

Subcellular Localization of GFP-SULT1C1 in Living HEK293 Cells. The imaging results suggested that the subcellular location of SULT1C1 in living HEK293 cells was cytoplasmic (Fig. 1), and the fluorescent signal of GFP-SULT1C1 was not associated with that of the nucleus. This is consistent with previous imaging studies with formaldehyde-fixed cell samples including our studies on SULT1E1, another member of the SULT family (Kapoor et al., 2007). The shared subcellular location between SULT1C1 and SULT1E1 also raises the question of whether different sulfotransferase isoforms interact with each other or form dimeric structures when they are

![Nucleus (Emission: 430-460 nm)](image1)

![GFP. SULT1C1 (Emission: 510 nm)](image2)

![Merge](image3)

**Fig. 1.** Expression of the GFP-tagged SULT1C1 proteins in living HEK293 cells. Human HEK293 cells were transfected with the pcDNA3.1/GFP-SULT1C1 vector. The transfected cells were enriched and maintained in their subculture medium containing 0.2 mg/ml G418. To measure the fluorescent signals of the transfected chimeric proteins, cells were excited at 488 nm by an argon laser (Olympus) and imaged with an Olympus FV300 confocal microscope (Olympus). The fluorescent signal of GFP-SULT1C1 was detected at 510 nm. Hoechst 33342, a fluorescent DNA dye, was used for the colocalization of GFP-SULT1C1 with the nucleus according to the manufacturer’s instructions.
expressed in the same subcellular compartment because sulfotransferase dimerization has been observed in previous in vitro crystallization studies (Negishi et al., 2001).

Mammalian cytosolic sulfotransferases were first isolated from the hepatic cytosol (Jakoby and Ziegler, 1990) and localized primarily in the cytoplasm as reported by previous cell imaging studies (Whitnall et al., 1993; Wang et al., 2004; Kapoor et al., 2007; Salman et al., 2009). There were discrepancies among these cell imaging studies on whether cytosolic sulfotransferases are also present in the nucleus and endoplasmic reticulum. It is also debatable whether the subcellular location of cytosolic sulfotransferases varies in different types of cells and whether it depends on the enzyme’s structural/functional status and interactions with other macromolecules including intracellular membrane systems.

With a set of 4551 human protein sequences for machine training, the subcellular location of SULT1C1 was assessed by machine learning methods in the present work. Results from two algorithms, SMO and MLP, showed that the most probable subcellular location for SULT1C1 is the cytosol and the second most probable location is the nucleus (Table 1). Similar results were obtained for the GFP-tagged SULT1C1 (Table 1). The predicted location of GFP was different between the two algorithms (Table 1). SMO predicted that the most likely subcellular location for GFP is the cytosol (probability 0.471), whereas MLP predicted that the nucleus (probability 0.884) is the most likely location for GFP.

Studies on the Cytoplasmic Mobility of GFP-SULT1C1 in Living HEK293 Cells with FRAP. Protein mobility is an indicator of protein membrane association and depends on the protein’s physicochemical properties and interactions with other macromolecules within the cell. In the present studies, FRAP was used to assess the mobility of SULT1C1 in living HEK293 cells. An argon laser (100% power, 4 min) was used to bleach the fluorescent signal of the expressed GFP-SULT1C1 in living HEK293 cells, and the fluorescent intensity was approximately 30% of the original level after bleaching. The fluorescent intensity within the area being bleached was gradually recovered with time (Fig. 3). The significance of the mobility of cytosolic sulfotransferases in living cells has not been established. The intracellular movement of cytosolic sulfotransferases may be important for maintaining the cellular homeostasis, activating or deactivating certain biological molecules or facilitating their intracellular transport.

The bleached fluorescent signal of GFP-SULT1C1 was not fully recovered at the longest time interval (10 min) monitored after photobleaching (Figs. 2 and 3). This result indicates that a fraction of the expressed GFP-SULT1C1 molecules would have much lower molecular mobility. The potential immobility of these GFP-SULT1C1 molecules might result from their attachment to certain subcellular membrane systems or macromolecules. Among the subcellular membrane systems, the endoplasmic reticulum was reportedly colocalized to the cytosolic sulfotransferase enzymes expressed in formalin-fixed cells (Whitnall et al., 1993). With the 4551 human protein sequences from SWISS-PROT as machine training sequences, machine learning
experiments indicated that the possibility of the association of SULT1C1 with the endoplasmic reticulum is protein aggregation, a concentration-dependent event that is related to the protein’s solubility. Cytosolic sulfotransferase aggregation has been reported previously (Wang et al., 2004), and recombinant protein overexpression itself might trigger such recombinant protein aggregation.

In summary, subcellular location and molecular mobility provide insights into the physiological roles of enzymes. In the present studies, we examined the subcellular location and molecular mobility of human cytosolic sulfotransferase 1C1 in living cells using GFP-tagging and confocal laser-scanning microscopy methods. We also assessed the probability of the subcellular location of SULT1C1 using machine learning methods based on the subcellular locations of 4551 previously annotated human proteins from the SWISS-PROT database. The cell imaging studies localized the GFP-SULT1C1 chimeric protein in the cytoplasm of living human HEK293 cells with no colocalization with the nucleus. Results from the machine learning studies were consistent with those from the cell imaging studies, but the association of cytosolic sulfotransferases with subcellular membrane systems still could not be excluded. FRAP studies suggested that a portion of the expressed GFP-SULT1C1 molecules was immobile in the cytoplasm of living HEK293 cells. More studies are needed to further explore the relationships between cytosolic sulfotransferases and subcellular membrane systems in living cells.

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Authorship Contributions

Participated in research design: Sheng and Acquaah-Mensah.
Conducted experiments: Sheng and Acquaah-Mensah.
Performed data analysis: Sheng and Acquaah-Mensah.
Wrote or contributed to the writing of the manuscript: Sheng and Acquaah-Mensah.

References