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Biochemical Pharmacology 66 (2003) 2089–2097
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Phenol sulfotransferase 1A1 activity in human liver: kinetic properties, interindividual variation and re-evaluation of the suitability of 4-nitrophenol as a probe substrate

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Received 3 June 2003; accepted 22 July 2003

Abstract

Sulfation is an important metabolic pathway in humans for xenobiotics, hormones and neurotransmitters, and is catalysed by the cytosolic sulfotransferase (SULT) enzymes. Phenol SULTs, especially SULT1A1, are particularly important in xenobiotic and drug metabolism because of their broad substrate specificity and extensive tissue distribution. A common variant SULT1A1 allozyme (SULT1A1*2) exists in the population, and is less stable than the wild-type SULT1A1*1. 4-Nitrophenol is widely used as a substrate for quantifying SULT1A1 activity. However, our kinetic experiments suggest that 4-nitrophenol is not an ideal substrate when determining SULT1A1 activity in human liver. Assays with a bank of 68 human liver cytosols revealed three distinct kinetic profiles for 4-nitrophenol sulfation in the population: linear, biphasic and inhibition. Sulfation of 4-nitrophenol by purified, recombinant SULT1A1*1 and SULT1A1*2 shows marked substrate inhibition, with inhibition at 4-nitrophenol concentrations greater than 4 and 10 μM, respectively. Furthermore, sulfation of 4-nitrophenol by purified recombinant SULT1B1 was significant at concentrations of 4-nitrophenol less than 10 μM. Western blots showed that the SULT1A1 levels in liver are highly variable between liver samples and that no correlation was observed between SULT1A1 activity and protein level in liver cytosols. However, a correlation between SULT1A1 activity and protein level was observed in human placental cytosols, where SULT1B1 is not expressed. We believe that in human liver other SULT isoforms (particularly SULT1B1) contribute to the sulfation of 4-nitrophenol. Therefore, 4-nitrophenol is not an ideal substrate with which to quantitate SULT1A1 activity in human liver tissue.

Keywords: Sulfotransferase; Drug metabolism; Enzyme kinetics; 4-Nitrophenol; Sulfation; Human liver

1. Introduction

Sulfation, catalysed by members of the cytosolic SULT enzyme family, is important for the homeostasis of endogenous compounds (e.g. catecholamines, steroid and thyroid hormones) and for the detoxification of xenobiotics, including many drugs and dietary constituents [1]. These enzymes catalyse the transfer of a sulfuryl moiety from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the acceptor substrate [1–3], generally leading to a decrease in biological activity and an increase in water solubility of the compound, thus aiding its excretion in urine and/or bile. The SULTs constitute a gene superfamily, which can be divided into a number of subfamilies based on their substrate specificity and amino acid sequence identity [1–3]. In humans 11 isoforms, encoded by 10 genes, have been identified to date. Phenol SULTs, in particular SULT1A1, are important in xenobiotic metabolism because of their broad substrate specificity and high affinity as well as their extensive distribution and abundance throughout the body [1,2].

Functional polymorphisms of phenol SULTs may influence individual susceptibility to adverse drug reactions involving drug substrates and also to chemical carcinogenesis [4–7]. Three human phenol SULTs (SULTs 1A1, 1A2 and 1A3) are encoded by neighbouring genes on chromosome 16 [8], and *SULT1A1* and *SULT1A2* display common

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Abbreviations: SULT, sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

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doi:10.1016/S0006-2952(03)00582-3

functional genetic polymorphisms [9–11]. A common variant allele of *SULT1A1*, called *SULT1A1*2*, exists at a frequency of approximately 32% in the Caucasian population. This variant codes for an allozyme (SULT1A1*2) with a single amino acid substitution ($Arg^{213} \rightarrow His$) compared with the wild-type SULT1A1*1. *SULT1A1*2* homozygotes possess approximately 10–15% of the platelet SULT enzyme activity associated with SULT1A1 compared with individuals homozygous for *SULT1A1*1* and it has recently been shown that the SULT1A1*2 allozyme is inherently unstable [9,12], resulting in decreased activity and protein levels.

The xenobiotic 4-nitrophenol has been widely used to estimate SULT1A1 activity in human tissues (e.g. [13,14]), and is generally accepted as a ''diagnostic'' or ''probe'' substrate. In this paper, we describe detailed kinetic experiments with purified recombinant SULTs and human liver cytosols using 4-nitrophenol as the substrate. Our results show that the quantification of SULT1A1 activity in human liver is not straightforward, and suggest that 4-nitrophenol is not an appropriate ''probe'' substrate for SULT1A1 as is commonly believed.

2. Materials and methods

2.1. Materials

Protein purification columns and media were purchased from Amersham Pharmacia Biotech. [^{35}S]PAPS was obtained from DuPont/NEN, PAPS (>99% purity) was purchased from H. Glatt, German Institute for Human Nutrition, Potsdam, Germany, and T-gel resin was from Pierce. Scintillation fluid (Emulsifier Safe) was from Canberra Packard. All other chemicals were from Sigma, BDH or Boehringer. Water used in all experiments was deionized and had conductivity <5.5 μS cm^{-1}.

2.2. SULT cDNA cloning and expression and purification of recombinant human SULTs

We have previously reported the cloning of cDNAs encoding SULTs 1A1*1 [15], 1A3 [16], 1E1 [17] and 2A1 [18]. SULTs 1A1*2, 1A2 and 1B1 were isolated by PCR from human liver cDNA (Quick-Clone™, Clontech) as described elsewhere [19]. PCR primers were designed to incorporate restriction sites (*Nde*I/*Xho*I for SULT1A1*2, *Nde*I/*Bam*HI for SULTs 1A2 and 1B1) to enable cloning into the pET17b *Escherichia coli* expression vectors (Novagen). Following PCR reactions, products were first ligated into pCR2.1 (Invitrogen) and sequenced on both strands to confirm identity. cDNAs were excised using the appropriate restriction enzyme pairs, ligated into pET17b and transformed into *E. coli*. The SULT2A1 cDNA was amplified from the pMPSV vector using PCR [18], ligated into pET17b as described above and sequenced.

SULT cDNAs were expressed in *E. coli* essentially as described previously [15,17,20]. For recombinant protein expression cDNAs encoding SULTs 1A1*1 (Arg^{213}), 1A1*2 (His^{213}), 1A3 and 2A1 were transformed into *E. coli* strain BL21(DE3)pLysS. SULTs 1B1 and 1A2 were expressed in *E. coli* strain BL21(DE3) and SULT1E1 (cloned in pCW) was expressed in *E. coli* strain JM109. For purification, bacterial cell-free extracts were subjected to 55% ammonium sulfate fractionation and, following dialysis, applied to a column of Q-Sepharose equilibrated with 50 mM Tris–HCl (pH 8.0) containing 1 mM mercaptoethanol. SULT protein was eluted with 600 mM NaCl, and fractions containing high activity were pooled, dialysed and applied to a 3′,5′-ADP agarose column equilibrated with 50 mM Tris–HCl (pH 8.0) containing 1 mM mercaptoethanol. After washing with 50 mM Tris–HCl (pH 8.0) containing 1 mM mercaptoethanol and 50 mM NaCl, the bound protein was eluted with 100 μM PAPS. Fractions containing SULT activity were pooled, desalted through a PD10 column and stored at −70°. All purification steps were undertaken at 4°.

2.3. Preparation of liver cytosols

Human liver cytosols and some liver tissue samples (previously frozen at −70°) were obtained from IIAM and from GlaxoSmithKline [21]. Ethical approval for use of liver samples was obtained at sites of collection and from the Tayside Committee on Medical Research Ethics. Of the samples where clinical information was available, the average age (±SD) of donor was 36 ± 17 years, range 1–67 years, and the ratio of males to females was 0.82. Liver tissue (previously frozen at −70°) was placed in ice-cold 10 mM HEPES (pH 7.4) containing 0.25 M sucrose and 3 mM 2-mercaptoethanol. The tissue was cut with scissors and homogenised (20% w/v) in a Teflon-glass homogenizer (Jencons Scientific Ltd). The homogenates were then centrifuged at 10,000 *g* for 15 min and the resulting supernatant was then subjected to further centrifugation at 100,000 *g* for 1 hr. The resulting cytosolic fraction was aliquoted and stored at −70°. Other cytosol samples obtained had been prepared essentially as described above, following homogenisation in 0.1 M Tris acetate, pH 7.4, 0.1 M potassium chloride, 1 mM EDTA and 20 μM butylated hydroxytoluene.

2.4. Enzyme assay

Sulfation of 4-nitrophenol was assayed according to the method originally described by Foldes and Meek [22]. Reaction mixtures in a final volume of 160 μL comprised 6.25 mM potassium phosphate buffer (pH 7.4), 60 μM PAPS containing 0.04 μCi [^{35}S]PAPS, 2 μg recombinant SULT enzyme or 75 μg liver cytosolic protein and 0.125–100 μM 4-nitrophenol, unless otherwise indicated. Blank reactions contained deionized water in place of the substrate or 6.25 mM potassium phosphate buffer (pH 7.4) in

place of the enzyme. Reactions were started by the addition of PAPS/[^{35}S]PAPS and incubated for 10 min at 37°. PAPS/[^{35}S]PAPS not utilised in the reaction was precipitated with 200 μL 0.1 M barium acetate, 200 μL 0.1 M barium hydroxide and 200 μL 0.1 M zinc sulfate and centrifuged for 5 min at 14,000 *g*. The supernatant (500 μL) was mixed with 3 mL of scintillation fluid and radioactivity quantified by liquid scintillation spectrometry. Assays were optimised for protein and incubation time, and enzyme inactivation during the assay was assessed. Data were analysed and kinetic parameters determined with the software packages Hyper.exe v.1.1 (J.S. Easterby, Department of Biochemistry, University of Liverpool, Liverpool, UK) and Prism v 3.0 (GraphPad Software Inc.).

2.5. Protein determination

The protein content of the liver cytosols and purified recombinant protein preparations was estimated using the Bradford's method [23], and BSA was used to develop a standard curve.

2.6. Immunoblot analysis

Purified recombinant SULT1A1 (50 and 100 ng) and human liver cytosol samples (5 μg) were resolved on a 12% SDS–PAGE mini-gel (Bio-Rad) and transferred to nitrocellulose membrane (Schleicher & Schuell). The membranes were blocked with 1% BSA overnight and incubated with sheep anti-SULT1A3 IgG [34], purified from antiserum using T-gel (Pierce) [24]. This antibody cross-reacts with SULTs 1A1, 1A2 and 1A3 because of the high degree of amino acid sequence identity (>93%) between these enzymes, however, these three isoforms are resolved on SDS–PAGE. Membranes were washed in TBS-X (50 mM Tris, pH 7.9, 150 mM NaCl, 0.1% (v/v) Triton X-100) and incubated with anti-sheep IgG peroxidase conjugate (Sigma) in TBS-X containing 1% BSA, washed in TBS-X, developed in ECL reagent and exposed to Hyperfilm ECL (Amersham). A standard curve was developed using increasing concentrations of purified recombinant SULT1A1 (0.05–1.25 μg), and immunoreactivity was quantified using Quantiscan 3.1 (BioSoft). Liver cytosols (5 μg) were analysed for SULT1A1 enzyme protein with two SULT1A1 standards within the linear region of the standard curve present on each blot. Correlation between activity and SULT protein level was assessed using Prism v 3.0 (GraphPad Software Inc.).

3. Results

3.1. Optimisation of SULT enzyme assay

The assay for SULT1A1 activity was optimised for incubation time, protein and PAPS concentrations. All assays (unless otherwise indicated) were incubated for 10 min with 2 μg per assay of recombinant protein or 75 μg per assay human liver cytosolic protein. No enzyme inactivation during the assay could be detected as determined by Selwyn's test [25,26], when the protein content of the assay was varied between 0.5 and 4 μg per assay with incubation times between 2 and 10 min (not shown). At concentrations of PAPS between 10 and 60 μM, there appeared to be no significant increase in inhibition with recombinant SULT1A1 or with liver cytosol. Furthermore, a correlation was found between specific activity (V_{max}) of SULT1A1 measured at PAPS concentrations of 20 and 60 μM, with a Pearson's *r*-value of 0.96.

3.2. Activity of SULT1A1 in human liver cytosol

Kinetic analysis was performed on 68 individual liver cytosols with 4-nitrophenol concentrations between 0.12 and 100 μM, and PAPS at 60 μM. Three distinct types of kinetic characteristics were observed within this sample group. Eighteen of the liver cytosols displayed linear Michaelis–Menten kinetics (Fig. 1A), 35 showed inhibition kinetics (Fig. 1B) and 15 displayed biphasic type kinetics (Fig. 1C). The maximal activity for the sulfation of 4-nitrophenol by each liver cytosol was determined using the V_{max} for samples displaying linear and biphasic kinetics, and by determining the maximum SULT activity before inhibition occurred for the inhibition kinetics. There was a large inter-sample variation in the maximal activity of 4-nitrophenol sulfation, with a range of 15–3044 pmol min^{-1} mg^{-1} (mean: 488; median: 415; SD: 414). No significant differences were observed in relation to age, gender, source of liver sample or preparation method.

3.3. Immunoblot analysis of SULT1A1 in human liver

Immunoblot analysis of liver cytosols showed a large variation in the level of SULT1A1 enzyme protein in human liver (a representative blot is shown in Fig. 2). However, these SULT1A1 enzyme protein levels did not appear to relate to the sulfation of 4-nitrophenol measured in kinetic experiments in each individual liver sample. Correlation analysis was performed to compare the maximum specific activity of 4-nitrophenol sulfation by 68 liver cytosols (determined by kinetic experiments) and the SULT1A1 expressed in each liver (determined by immunoblot analysis). No significant correlation was found, with a Pearson's *r*-value of 0.28 (Fig. 3). To determine whether this phenomenon was restricted to the liver, the specific activity and SULT1A1 protein level in a group of placental cytosols was determined under identical conditions and correlation analysis performed. A strong correlation was found between the sulfation of 4-nitrophenol (V_{max}) and level of SULT1A1, with a Pearson's *r*-value of 0.82 (Fig. 3), confirming previous observations with placental SULT1A1 [14].

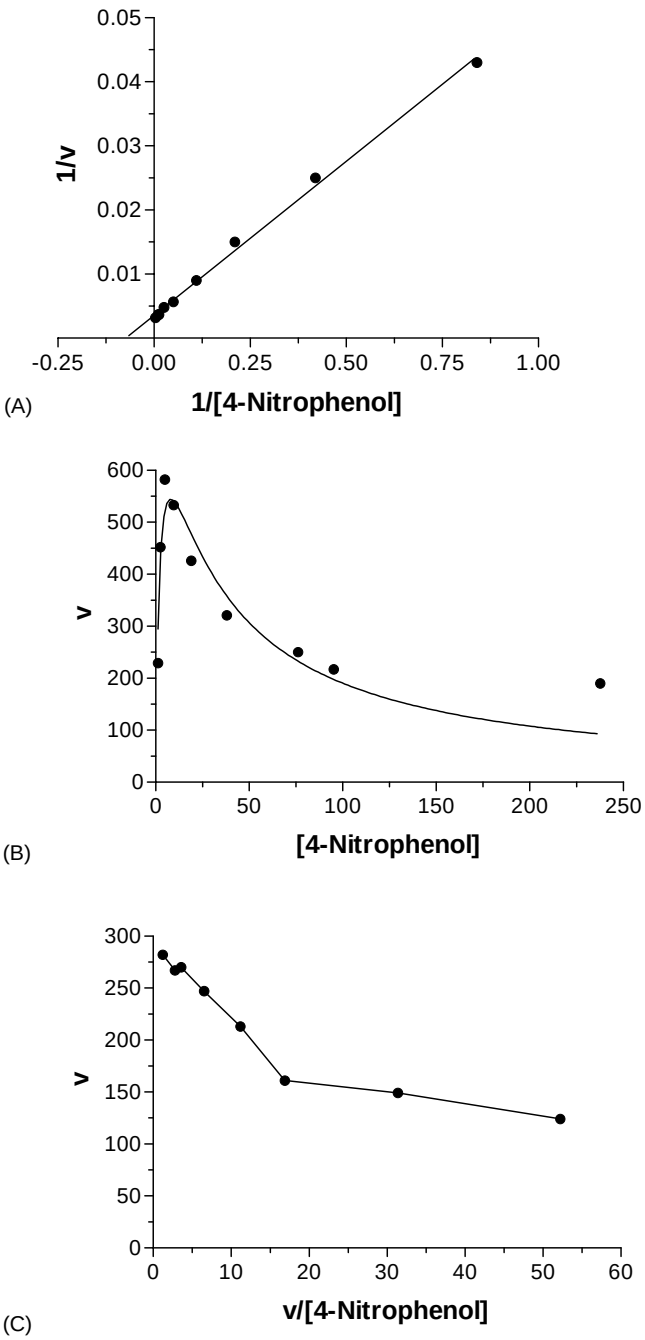

Fig. 1. Kinetic profiles of the sulfation of 4-nitrophenol by human liver cytosols reaction mixtures were of the composition described, with the concentration of 4-nitrophenol varied between 0.125 and 100 μM and PAPS concentration held at 60 μM. Activity (v) is in pmol min^{-1} mg^{-1} protein. The data are an average of duplicates. Graphs A, B and C are representative of the three distinct kinetic profiles observed across the 68 liver cytosol samples.

3.4. Kinetic characteristics of purified recombinant SULT1A1*1 and SULT1A1*2

Kinetic plots with purified recombinant SULT1A1*1, with PAPS at 60 μM and 4-nitrophenol concentrations varied between 0.125 and 100 μM, showed inhibition at 4-nitrophenol concentrations greater than 2 μM (Fig. 4). At high concentrations of 4-nitrophenol the specific activity of SULT1A1*1 did not approach zero, suggesting partial substrate inhibition. The data for 4-nitrophenol sulfation by SULT1A1*1 were analysed using the equation for partial substrate inhibition ($v = (V_1 s + V_2 s^2)/K_m + s + (s^2/K_i)$) (Table 1). The kinetic data for the sulfation of 4-nitrophenol by SULT1A1*2 also displayed partial substrate inhibition; however, inhibition only occurred at 4-nitrophenol concentrations greater than 10 μM (Fig. 4, Table 1).

The activity of purified, recombinant SULT1A1*1 was measured at five concentrations of 4-nitrophenol and five

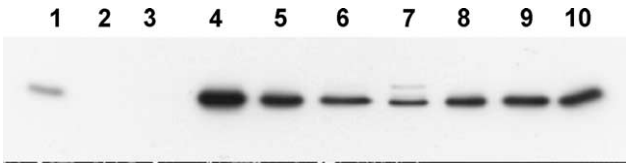

Fig. 2. SULT1A1 in human liver. The expression of SULT1A1 (5 μg) in human liver cytosol was estimated by immunoblot analysis as described in the Section 2. Purified recombinant SULT1A1 (20 and 40 ng) is in lanes 1 and 10, respectively. In lanes 2–9, 5 μg of liver cytosolic protein was applied.

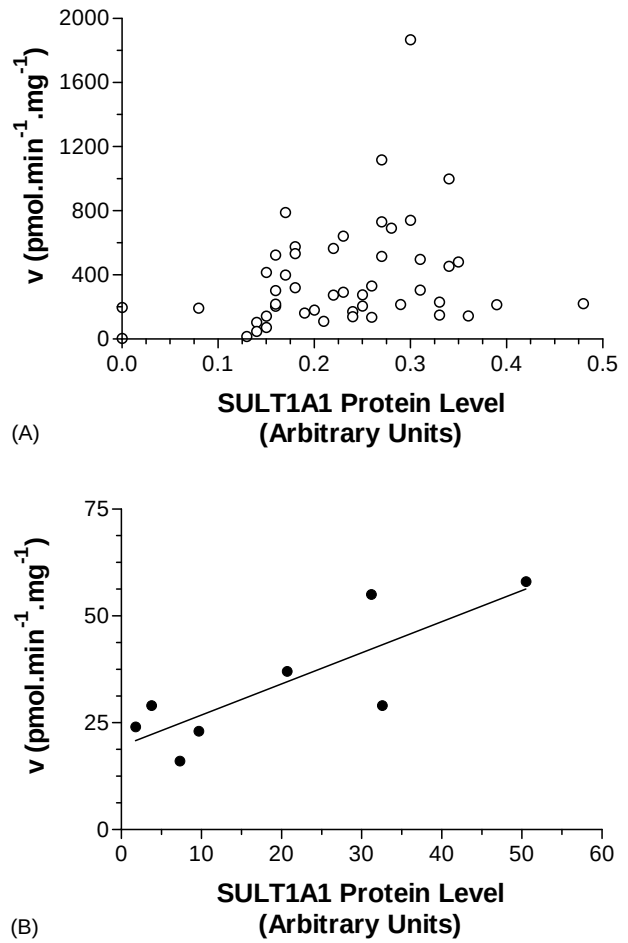

Fig. 3. Correlation analysis of the sulfation of 4-nitrophenol and level of SULT1A1 in human liver (A) and placental (B) cytosol. The sulfation of 4-nitrophenol was determined by kinetic studies (V_{max}) and the SULT1A1 protein level was determined by immunoblot analysis, as described in the Section 2.

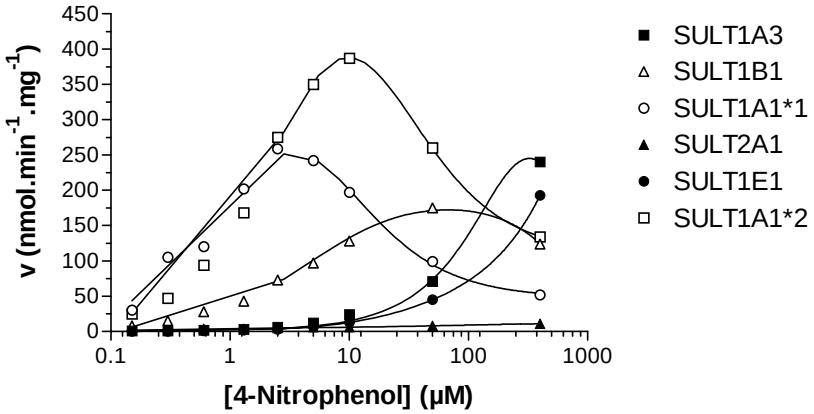

Fig. 4. Activity of purified recombinant SULTs under assay conditions. The sulfation of 4-nitrophenol by purified recombinant SULTs 1A1*1, 1A1*2, 1A3, 2A1, 1B1 and 1E1 under the conditions of our assay was examined. PAPS concentration was held at 60 μM. The data are representative of the average of duplicates from three replicate experiments.

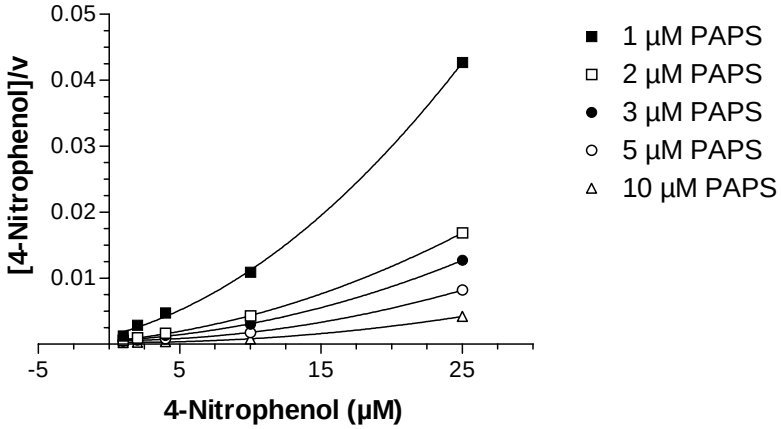

Fig. 5. Inhibition of SULT1A1*1 activity by 4-nitrophenol. Kinetic properties of purified recombinant SULT1A1. Reaction mixtures were of the composition described in the Section 2, with 4-nitrophenol and PAPS concentrations as indicated. Activity (v) is in pmol min^{-1} mg^{-1} protein. The data are representative of an average of duplicates from three replicate experiments.

concentrations of PAPS (Fig. 5). This experiment demonstrates and confirms substrate inhibition by 4-nitrophenol and suggests that catalysis by SULT1A1 proceeds *via* a complicated ternary-complex mechanism.

3.5. Sulfation of 4-nitrophenol by other SULT isoforms in liver

In order to assess the potential contribution of other SULT isoforms to the sulfation of 4-nitrophenol in human liver cytosol, sulfation of 4-nitrophenol by purified recombinant SULTs 1A3, 2A1, 1B1 and 1E1 was determined under assay conditions identical to those used for SULT1A1 (Fig. 4). Other known SULT isoforms (1C2, 1C4, 2B1, 4A1) are not expressed in adult human liver [27–30]. At 4-nitrophenol concentrations below 4 μM, SULT1B1 catalysed the sulfation of 4-nitrophenol at significant levels, whereas SULT1A3 and SULT1E1 only sulfated this substrate at concentrations greater than 50 μM. SULT2A1 did not sulfate 4-nitrophenol under the assay conditions used.

We also attempted to find a substrate that might be more specific for SULT1A1 and that did not display partial substrate inhibition, as does 4-nitrophenol. We have previously screened more than 90 phenols and catechols for sulfation by various human SULTs [19,31]. These studies suggested some possible substrates, and sulfation of phenol, 4-phenylphenol, 4-ethylresorcinol, 2,3-dihydroxybenzoic acid, 4-isopropylphenol, 4-methylphenol and 4-isopropyphenol by SULT1A1 was examined further. 4-Phenylphenol appeared reasonably selective for SULT1A1, and using the

Table 1
Kinetic constants of purified recombinant SULT1A1*1 and SULT1A1*2

Kinetic constants	SULT1A1*1	SULT1A1*2
K_m (μM)	5.5 ± 0.04	2.9 ± 0.5
V_1 (nmol min^{-1} mg^{-1})	386 ± 86.5	671 ± 69.6
V_2 (nmol min^{-1} mg^{-1})	49.4 ± 28.4	18.4 ± 5.9
K_i (μM)	1.7 ± 0.1	18.4 ± 5.9

Reaction mixtures were of the composition described in the Section 2, with 4-nitrophenol and PAPS concentrations as indicated. The data ± SE are an average of duplicates from three replicate experiments.

purified enzyme and a subset of liver cytosols (representative of the three kinetic types with 4-nitrophenol as substrate), no substrate inhibition with 4-phenylphenol was observed. However, the turnover of this substrate was very low (i.e. low V_{max}) and so 4-phenylphenol was not a suitable substrate for liver samples exhibiting low to moderate SULT1A1 activity. No other substrate tested fulfilled these criteria.

4. Discussion

Phenol SULT activity has been demonstrated in many human tissues [2,14,32,33]. In adult liver phenol SULT activity towards 4-nitrophenol is high, although extremely variable. Expression of SULTs 1A1, 1B1, 1E1 and 2A1 in adult human liver is well documented and characterised. SULT1A3 protein is not expressed in adult liver at any appreciable level [34], and it is questionable whether SULT1A2 protein is functionally expressed in any tissue [35]. The measurement of phenol SULT activity is generally determined using dopamine as the substrate for SULT1A3 and 4-nitrophenol as the substrate for SULT1A1 (and SULT1A2), based on the high affinity of these enzymes for the respective substrates [15]. However, the specificity of these enzymes is not absolute, and other SULT isoforms can catalyse the sulfation of these substrates. The combined sulfation capacity of differentially expressed multiple SULT isoforms may explain the three different kinetic characteristics of 4-nitrophenol profiles observed in human liver.

X-ray crystallographic studies on a number of isoforms confirm that overall structures of the SULT family are well conserved [31,36–40]. Elegant crystallographic and mutational studies on mouse SULT1E1 by Negishi and colleagues suggest a compulsory ordered mechanism, and indicated that a ternary-complex is formed during catalysis in which the substrate is situated in a hydrophobic pocket near the PAP binding site [41]. Experiments with rhesus monkey liver bile salt SULT, human brain SULT1A family and plant flavanol SULTs suggest an ordered bi bi mechanism [42–44]. However, studies with human SULT1E1 suggested a random bi bi mechanism with two dead end complexes formed during catalysis [45]. Kinetic studies with rat liver SULT1A1 also suggest that catalysis follows a random bi bi mechanism in which PAPS and 4-nitrophenol bind to the enzyme independently [46]. In our experiments partial substrate inhibition was observed in kinetic studies of recombinant human liver SULT1A1*1 with 4-nitrophenol as acceptor substrate, as demonstrated by the Hanes plot shown in Fig. 5. Here the plots of [4-nitrophenol]/v against [4-nitrophenol] are parabolic with a common intersection point for the second binding substrate (i.e. PAPS) [47]. These kinetic experiments also indicate that catalysis proceeds *via* a complicated ternary-complex mechanism.

Recently, further light was shed on the reaction mechanism of SULT1A1 with the publication of the X-ray crystal structure of the enzyme [40]. These studies showed that (at least in crystal form) SULT1A1 can bind two molecules of 4-nitrophenol, providing an explanation for the substrate inhibition with this substrate observed *in vitro*. It should be noted, however, that the structure reported by Gamage *et al*. [40] was in fact that of the SULT1A1*2 allozyme, which we demonstrate here displays considerably less substrate inhibition than the SULT1A1*1 allozyme (Table 1).

The common variant allozyme of SULT1A1 (SULT1A1*2) differs from SULT1A1*1 by a single amino acid substitution ($Arg^{213} \rightarrow His$) [10,48], and the reduced SULT1A1 enzyme protein observed in individuals homozygous for the *SULT1A1*2* allele appears to be due to the reduced biological half-life of the protein [12]. Homozygous or heterozygous expression of SULT1A1*1 and SULT1A1*2 could influence the apparent activity of SULT1A1 in human liver. Our data show that the K_m of SULT1A1*2 for 4-nitrophenol is lower than for SULT1A1*1, and also that SULT1A1*2 is less susceptible to substrate inhibition, with a K_i for the inhibition more than 10 times greater. The relative specificity constant (V_{max}/K_m) for 4-nitrophenol sulfation by SULT1A1*1 at non-inhibitory concentrations is lower than that of SULT1A1*2 (70.2 and 213.4, respectively). However, at inhibitory concentrations of 4-nitrophenol, the value for SULT1A1*1 is higher than SULT1A1*2 (9.0 and 2.2, respectively). Therefore, it is possible that both isoforms could contribute significantly to the sulfation of 4-nitrophenol in human tissues, although the SULT1A1*2 protein may be present at much lower levels due to its apparent reduced half-life.

SULT protein in liver cytosol, determined by Western blotting, indicated that SULT1A1 varied greatly between individuals. However, when the SULT1A1 protein level was compared to maximum 4-nitrophenol sulfation activities of the liver cytosols (i.e. estimated V_{max}), no correlation was observed. This is in contrast to other human tissues, such as platelets [49], placenta (Fig. 3, [14]) and foetal liver [34], where good correlation between SULT1A1 enzyme activity (measured with 4-nitrophenol) and enzyme protein level has been observed. SULTs 1B1 and 1E1 are not expressed to any significant level in placenta [14] nor in platelets, and foetal liver shows negligible SULT1B1 protein although SULT1E1 is high [30].

To date, no specific substrate for SULT1B1 is known. Kinetic studies reported here reveal that sulfation of 4-nitrophenol by SULT1B1 is not subject to substrate inhibition as with SULT1A1, and also that the relative specificity constant of SULT1B1 for 4-nitrophenol was 56.7, higher than for both SULT1A1*1 and SULT1A1*2 at 4-nitrophenol concentrations which inhibit these two allozymes. This suggests that the contribution of 4-nitrophenol sulfation by SULT1B1 is dependent on the substrate concentration

and the relative levels of SULT1A1*1, SULT1A1*2 and SULT1B1 in the liver. For example, if the levels of SULT1A1*1, SULT1A1*2 and SULT1B1 were equimolar, at a concentration of 4-nitrophenol of 2.5 μM (i.e. below inhibitory concentrations for SULT1A1), the calculated relative contributions to total 4-nitrophenol SULT activity would be approximately 43, 45 and 12%, respectively. However, at a concentration of 4-nitrophenol inhibitory for SULT1A1*1 and SULT1A1*2 (e.g. 50 μM), then the predicted contribution to total activity would be 18% by SULT1A1*1, 49% by SULT1A1*2 and 33% by SULT1B1. Therefore, the varied kinetics observed with 4-nitrophenol sulfation in liver cytosols could be a result of additive contributions to sulfation by SULT1A1*1, SULT1A1*2 and SULT1B1, and the different kinetic behaviour is unlikely to be due to low molecular weight factors effecting activity but more likely attributable to the variable levels of SULT1B1. A further complication could be introduced by the occurrence of heterodimers of SULT1A3 and SULT1A1 in tissues which may give rise to active forms of SULT1A, with properties distinct from the two individual SULT1As [2]. The formation of heterodimers between SULTs 1A1 and 1C1 has been demonstrated in rat liver cytosol [50] and with recombinant SULTs 1E1 and 2A1 [51]. However, this is unlikely, as SULT1A3 is essentially not expressed in healthy adult liver. It has been shown previously [14,30,34] that the antibody used for quantifying SULT1A1 in our experiments also recognises SULTs 1A3 and 1A2, which can be resolved from SULT1A1 during SDS–PAGE. There was no evidence to suggest that either SULT1A2 or SULT1A3 were expressed in human liver, as has been shown by others (e.g. [35]).

Variable levels of SULT isoforms in individuals poses an interesting problem when determining the relative roles and contributions to xenobiotic sulfation of each isozyme. Variation in *SULT1A1* allele frequencies within and between populations has been documented [10,11,52]. The tissue in which the SULT is expressed may also influence the activity by the exposure to ''external'' influences, such as nutritional factors (e.g. a high fat diet may alter SULT activity in the colon [53]). The quantification of a given isoform of SULT in human liver is difficult because of the non-specificity of known substrates, high amino acid sequence identity and variation in isoform levels throughout the population.

5. Conclusion

The kinetic experiments reported here strongly suggest that 4-nitrophenol is not an ideal substrate for the determination of SULT1A1 activity in human liver cytosol. The contribution of several isoforms and/or allozymes to the measured activity may account for the lack of correlation between SULT1A1 activity and protein level in adult human liver tissue. This was further substantiated by a correlation observed in tissues that do not express many other SULTs (e.g. placenta). Sulfation of 4-nitrophenol in human liver cytosol could be catalysed by SULTs 1A1*1, 1A1*2 and/or 1B1, depending on their relative levels within a particular liver. The interindividual variation in levels of SULTs 1A1*1, 1A1*2 and 1B1 in liver tissue may account for the three different kinetic characteristics observed across the 68 liver samples. A suitable specific substrate for quantifying SULT1A1 activity in human liver cytosols remains to be found, and we strongly recommend that enzyme activity data generated with 4-nitrophenol should be interpreted with caution.

Acknowledgments

We are grateful to Drs. Alan Hood and Rana Dajani for cloning and expression of SULT1A1*2, SULT1A2 and SULT1B1 cDNAs. Protein purification was carried out by Nerys Thomas, Laura Johnston and Nicola Rose. We express our gratitude to Dr. Tim Mantle (Trinity College, Dublin) for his valuable help with the kinetic analysis, and to Professor Jyrki Taskinen (University of Helsinki) and Dr. Ann Gordon for critical reading of the manuscript and helpful suggestions. This work was funded by the Human Drug Conjugation Consortium (AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, F. Hoffman-La Roche, Lilly, Novartis, Pfizer, Wyeth-Ayerst), and in part by the Commission of the European Communities (BMH4-CT97-2621).

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