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Reaction product affinity regulates activation of human sulfotransferase 1A1 PAP sulfation[★]

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Abstract

Cytosolic sulfotransferase (SULT)-catalyzed sulfation regulates the activity of bio-signaling molecules and aids in metabolizing hydroxyl-containing xenobiotics. The sulfuryl donor for the SULT reaction is adenosine 3'-phosphate 5'-phosphosulfate (PAPS), while products are adenosine 3',5'-diphosphate (PAP) and a sulfated alcohol. Human phenol sulfotransferase (SULT1A1) is one of the major detoxifying enzymes for phenolic xenobiotics. The mechanism of SULT1A1-catalyzed sulfation of PAP by pNPS was investigated. PAP was sulfated by *para*-nitrophenyl sulfate (pNPS) in a concentration-dependent manner. 2-Naphthol inhibited sulfation of PAP, competing with pNPS, while phenol activated the sulfation reaction. At saturating PAP, a ping pong kinetic mechanism is observed with pNPS and phenol as substrates, consistent with phenol intercepting the E–PAPS complex prior to dissociation of PAPS. At high concentrations, phenol competes with pNPS, consistent with formation of the E–PAP–phenol dead-end complex. Data are consistent with the previously reported mechanism for sulfation of 2-naphthol by PAPS, and its activation by pNPS [14]. Overall, data are consistent with release of PAP from E–PAP and PAPS from E–PAPS contributing to rate-limitation in both reaction directions.

Keywords

Human; Sulfotransferase; Kinetic mechanism; Initial velocity studies

Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes that catalyze the sulfation (sulfonation) of various hydroxyl-containing compounds [1–8]. Sulfation of xenobiotics is associated with detoxification, biotransformation of a relatively hydrophobic xenobiotic into a more water-soluble sulfuric ester that is readily excreted. SULTs also catalyze the sulfation of bio-signaling molecules such as hydroxysteroid hormones, thyroid hormones, glucocorticoid hormones, bile acids, and neurotransmitters. One of the main biological functions of SULTs is regulation of various hormones [9].

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¹Abbreviations used: SULT, sulfotransferase; SULT1A1, simple phenol sulfotransferase; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; PAP, adenosine 3',5'-diphosphate; pNPS, *para*-nitrophenyl sulfate.

Sulfation proceeds as shown below, where the sulfonyl group donor is adenosine 3'-phosphate 5'-phosphosulfate (PAPS), and the reaction products are adenosine 3',5'-diphosphate (PAP) and a sulfated product.

Studies of the kinetic mechanisms of SULTs began to appear in the 1980s [10]. Although many SULT isoforms have been isolated and characterized, their kinetic mechanisms have not yet been clearly defined. Substrate inhibition by the hydroxyl substrate is a common feature of most cytosolic SULTs [11,12].

Human phenol sulfotransferase (SULT1A1) is one of the major detoxifying enzymes for phenolic xenobiotics. It is widely distributed in human tissues. It has very broad substrate specificity and high catalytic activity toward most phenolic compounds. It not only sulfates simple xenobiotic phenols with high activity but also sulfates many endogenous phenolic molecules, including hormones and neurotransmitters. Sulfation of xenobiotic phenols will lead to metabolism and detoxification of the toxicants. Sulfation of endogenous bio-signaling molecules by SULT regulates their biological activity. Sulfation of hormones also increases their solubility and increases the transport of hormones in the blood to target tissues. Sulfation can also be used for stabilization and storage for some bio-signaling molecules.

Previously, on the basis of isotopic exchange at equilibrium, we determined that the SULT1A1 kinetic mechanism is steady state ordered with PAPS binding to the protein first and PAP released last [13]. Then, by studying activation of SULT1A1-catalyzed 2-naphthol sulfation by the product *para*-nitrophenyl sulfate (pNPS) we proposed a bypass mechanism with sulfation of PAP by pNPS prior to its release from the E-PAP complex, [14]. A ternary complex formed between substrate and the enzyme-PAP complex appears to be the source of substrate inhibition [14]. Substrate/product ability to activate or inhibit sulfation appears to depend on its affinity for the E-PAP complex. Low affinity pNPS activated sulfation through the bypass mechanism, while high affinity 2-naphthol inhibited it.

The sulfated hormones can be de-sulfated (re-activated) at target tissues when they are needed. It is known that SULTs also catalyze the reverse reaction. The catalytic mechanism of the SULT-catalyzed non-physiologic reverse reaction has not been studied to a significant extent. Although activation of the reaction by pNPS in the direction of the sulfated product has been proposed, the possibility of a similar activation on the direction of PAPS formation has not been considered. There is no guarantee that activation of the non-physiologic reaction will occur as a result of intercepting the E-PAPS complex prior to dissociation of PAPS. This would require that PAPS release contribute to rate limitation in the reverse reaction direction. To obtain more evidence on the proposed bypass mechanism and its causation in terms of product affinity we investigated the SULT1A1-catalyzed sulfation of PAP, which is the reverse of 1. Data would also provide information concerning the location of rate-limiting step(s) in both reaction directions.

This work demonstrated that the human SULT1A1-catalyzed reverse reaction shares kinetic features similar to those found in the forward reaction direction. Findings are important overall for an understanding of the potential biological function(s) that SULTs play. Previously, evidence was presented for the transfer of sulfate to other phenols, activated by PAP [15]. For the aryl sulfotransferase IV from male rat liver it was shown that high affinity

products, 1- and 2-naphthols, inhibited PAP sulfation, while phenol acted as a very poor inhibitor [10]. Data are discussed in terms of the overall reaction mechanism of SULT1A1.

Materials and methods

Chemicals

2-Naphthol was purchased from Fluka BioChemika, potassium 4-nitrophenyl sulfate (pNPS), adenosine 3',5'-diphosphate sodium salt (PAP), and phenol were purchased from Sigma–Aldrich. Deionized water was used in all the experiments. All the other reagents and chemicals were of the highest available grade.

Enzyme

SULT1A1 used in this study (7.5 mg/mL, maltose fusion protein) was purified as described previously [16–18].

pNPS enzyme activity assay

The pNPS assay takes advantage of the fact that phenol sulfotransferases (SULTs) catalyze the transfer of the sulfonyl group of pNPS to PAP, to give PAPS and a colored product, *p*-nitrophenolate (pNP), with maximum absorbance at 401 nm. Thus, the extent of the SULT1A1-catalyzed PAP sulfation can be monitored spectro-photometrically by the appearance of pNP. The ϵ_{401} for pNPS is $18.4 \text{ mM}^{-1} \text{ cm}^{-1}$ at pH values higher than 8. Reactions in these studies were carried out at pH 6.2 where the ϵ_{401} is much lower. A continuous assay would be difficult given the low K_m values for reactant. The % of substrate conversion per assay was typically 10–15% of the initial substrate concentration.

Reactions were carried out in 5 mL Pyrex test tubes; the total reaction volume was 250 μL . The test tubes with different initial concentrations of PAP, pNPS, and 2-naphthol or phenol where applicable, in 50 mM phosphate buffer (pH 6.2), were incubated at 37 °C for 2 min in an ORS 200 Boekel/Grant water bath. 2-Naphthol solutions were prepared by dissolving 2-naphthol in 100% ethanol. 2-Naphthol (5 μL) solution at the desired concentration was added to the test tube where applicable (2% ethanol by volume). Then, 5 μL of 50 mM phosphate buffer (pH 6.2) containing SULT1A1 was added to the reaction mixture to start the reaction. The test tubes were covered with aluminum foil to prevent evaporation. After the reaction was started, the test tubes were incubated for 30 min at 37 °C (the reaction time course is linear over this time). Then, the reactions were stopped with 250 μL of Tris base (0.25 M, pH 8.7). The absorbance at 401 nm was measured after the reaction was stopped using a Perkin-Elmer Lambda Bio UV/Vis spectrometer. Plastibrand semi-micro disposable cuvettes (1.5 mL) were used. Measurements were carried out in triplicate, and the average of the absorbance measurements minus the control was used to calculate the enzymatic activity. The controls were carried out in the absence of SULT1A1. The absorbance of controls at 401 nm was low, within the 0.04–0.08 range A_{401} .

Data fitting

All data were fit to the appropriate rate equation using the Enz-Fitter program from BIOSOFT, Cambridge, UK. Data shown in Figs. 1 and 3 were fitted to Eq. (2). Data for saturation curves shown in Fig. 2 were fitted to Eq. (1).

$$v = \frac{VA}{K_a + A} \quad (1)$$

$$v = \frac{VAB}{K_a B(1 + \frac{B}{K_{IB}}) + K_b A + AB} \quad (2)$$

In Eqs. (1) and (2), v and V are initial and maximum rates, respectively, K_a and K_b are Michaelis constants for A and B, respectively, A and B are reactant concentrations, and K_{IB} is a substrate inhibition constant.

Results

Reverse reaction kinetics

Sulfotransferase 1A1 catalyzes sulfation of PAP by pNPS. An initial velocity pattern obtained by measuring the rate of appearance of *p*-nitrophenol (pNP) as a function of pNPS at different fixed levels of PAP is shown in Fig. 1, and kinetic parameters are given in Table 1. The initial velocity pattern exhibits parallel lines at low pNPS, with substrate inhibition at high pNPS that is competitive *versus* PAP. There is evidence of an additional inhibitory effect at concentrations of pNPS higher than those shown in Fig. 1 (data not shown); the effect likely results from combination of pNPS to E-PAPS.

Effect of phenol and 2-naphthol

It was previously shown that activation of the SULT1A1-catalyzed sulfation of 2-naphthol by PAPS is observed in the presence of pNPS [14]. The activation was proposed to result from interception of the E-PAP complex, converting it to E-PAPS. In order to test whether the reverse reaction could also be activated, phenol and 2-naphthol were tested as potential activators of the reverse reaction.

A double reciprocal plot of velocity *versus* pNPS at a fixed concentration of PAP is shown in Fig. 2, and the same curve in the presence of 0.1 mM 2-naphthol exhibits apparent competitive inhibition *versus* pNPS. K_{pNPS} increases from $75 \pm 1 \mu\text{M}$ in the absence of 2-naphthol to $3.0 \pm 0.2 \text{ mM}$ in the presence of 2-naphthol, while V/E_t values of $0.0175 \pm 0.0003 \text{ min}^{-1}$ and $0.0185 \pm 0.0003 \text{ min}^{-1}$ are obtained, respectively.

A K_i of $2.6 \pm 0.2 \mu\text{M}$ for 2-naphthol can be obtained from the expression of $\text{app}K_{pNPS}$ ($K_{pNPS}[1 + [2\text{-naphthol}]/K_{i2\text{-naphthol}}]$). On the other hand, 0.1 mM phenol exhibits an increase in V/E_t from $0.0175 \pm 0.0003 \text{ min}^{-1}$ in the absence of phenol to $0.0684 \pm 0.0017 \text{ min}^{-1}$ in the presence of phenol, about a 4-fold activation. Essentially no change in K_{pNPS} is observed with values of $75 \pm 1 \mu\text{M}$ in the absence of phenol, compared to the value of $50 \pm 1 \mu\text{M}$ in the presence of phenol. Thus, although phenol activates the sulfation reaction, 2-naphthol inhibits.

An initial velocity pattern, obtained by measuring the initial rate as a function of pNPS and different fixed concentrations of phenol and a fixed concentration of PAP (0.1 mM) is shown in Fig. 3. In the absence of phenol the reaction occurs in agreement with data in Figs. 1 and 2. At low phenol concentrations the reaction rate increases and a parallel initial velocity pattern is observed, suggestive of a mechanism that mimics ping pong. At high concentrations of phenol inhibition is observed, evidenced by an increase in the slope of the reciprocal plot. Intersection of all of the lines at high concentration of phenol at a single point on the ordinate is indicative of competitive inhibition by phenol *versus* pNPS. A fit of the data to Eq. (2) gives the kinetic parameters in Table 2.

If one repeats the experiment shown in Fig. 3 at different concentrations of PAP, the rate is also increased since PAP is a substrate for the reaction. This is similar to the behavior of

PAPS and 2-naphthol in the presence of pNPS in the forward reaction direction, where PAPS and pNPS activate the sulfation of 2-naphthol [14].

Discussion

Kinetic mechanism of pNPS sulfation of PAP

As reported previously, the kinetic mechanism of the SULT1A1-catalyzed sulfation of 2-naphthol by PAPS is ordered with PAPS and PAP binding to free enzyme, and with uncompetitive substrate inhibition by 2-naphthol as a result of a dead-end E-PAP-2-naphthol ternary complex [13,14]. In order to determine whether the ordered mechanism holds in the reverse reaction direction, kinetic studies were carried out with PAP and pNPS as substrates.

Using pNPS as the sulfonyl donor in the direction of PAPS formation, the initial velocity pattern exhibits near parallel lines at low pNPS with substrate inhibition by pNPS *versus* PAP. Although the mechanism is steady state ordered with PAPS binding to the protein first and PAP released last as shown by isotopic exchange at equilibrium experiments [13], parallel lines are observed because $K_{\text{PAP}} > K_{\text{iPAP}}$ and under these conditions the constant term in the rate equation for an ordered mechanism ($K_{\text{iPAP}}K_{\text{pNPS}}$) tends to zero giving an equation identical to that obtained for a ping pong mechanism. As suggested in Results, there is evidence of noncompetitive substrate inhibition at higher pNPS concentrations, suggesting combination with E and E-PAPS. The combination of pNPS with E-PAPS exhibits a lower affinity than for E-PAP as expected since both PAPS and pNPS have the sulfonyl group to be transferred, and charge-charge repulsion, steric repulsion, or both, will result from binding of both in the same site.

Effects of phenol and 2-naphthol

A 4-fold activation of PAP sulfation is observed by phenol in the presence of pNPS, while 2-naphthol behaves as a competitive inhibitor with relatively high affinity. Do these two reactants produce their effect as a result of binding to the same enzyme form? Activation by phenol is uncompetitive, i.e. it has an effect on V/E_t only (V-type activator), and must bind to the E-PAPS complex. These data would require that release of PAPS from the E-PAPS complex is slow, and it must accumulate in the steady state. The competitive inhibition by 2-naphthol *versus* pNPS, suggests it binds to the same enzyme form as pNPS, E-PAP. Thus, either 2-naphthol does not bind to E-PAPS with significant affinity, but binds to E-PAP with high affinity, or it does bind to E-PAPS but does not contribute to a decrease in the off-rate constant of PAPS from E-PAPS at the concentration (0.1 mM) of 2-naphthol used. Data obtained previously indicate the K_m for 2-naphthol in the PAPS sulfation reaction is 12 μM , and although this is not a K_d it does suggest 2-naphthol has significant affinity for E-PAPS, and that release of PAPS from E-PAPS likely limits V/E_t in this reaction direction [14].

The reactant 2-naphthol binds to E-PAP as shown by uncompetitive substrate inhibition *versus* E-PAPS in the direction of naphthyl sulfate production [14]. In addition, 2-naphthol also exhibits competitive inhibition *versus* pNPS in the direction of PAPS formation, Fig. 2. In the direction of PAP formation, uncompetitive substrate inhibition by 2-naphthol results from binding to the E-PAP complex. The K_I value for the substrate inhibition will depend on the concentration of E-PAP that builds up in the steady state, with the true K_i value observed if release of PAP is completely rate limiting at saturating reactant concentrations, i.e. 100% of the enzyme is E-PAP. The K_I will increase with decreasing amounts of E-PAP in the steady state. The fractional amount of E-PAP compared to E_t that builds up in the steady state in the direction of PAP production can be determined from the ratio of the K_i for binding to E-PAP, $1.7 \pm 0.2 \mu\text{M}$, and its K_I for uncompetitive substrate inhibition, 110 ± 10

μM . On the basis of these data, about $1.5 \pm 0.2\%$ of the total enzyme is present as E–PAP at saturating concentrations of 2-naphthol and PAPS in the direction of naphthyl sulfate production. Thus, other steps along the reaction pathway, including the sulfonyl transfer step and/or release of naphthyl sulfate must contribute to rate limitation. In agreement with the low percentage of the E–PAP complex in the steady state, the addition of PAPS to a reaction containing PAP, pNPS, and an activating concentration of phenol increases the reaction rate as a result of increasing the amount of the E–PAPS complex (data not shown).

Activation by phenol likely results from an interception of the E–PAPS complex produced by sulfation of PAP by pNPS on enzyme, resulting in sulfation of phenol and regenerating E–PAP, Scheme 1. This interpretation would be consistent with previous results that suggested activation of the sulfation of 2-naphthol by PAPS on enzyme resulting from the interception of the E–PAP complex, which was sulfated by pNPS to regenerate E–PAPS [14]. In agreement, Fig. 3 corroborates activation by phenol that is uncompetitive *versus* pNPS, as a result of forming a E–PAPS–phenol complex. Increasing the concentration of PAP has a similar effect as a result of increasing the amount of the E–PAP complex in the steady state.

Why does 2-naphthol inhibit, while phenol activates?

The ability of a hydroxylated reactant to activate the sulfation of PAP by pNPS appears to be related to the second order rate constant for conversion of E–PAPS–ROH to E–PAP and ROSO_3^- . Consider binding of alcohol to the product E–PAPS complex in Scheme 1. If the alcohol becomes sulfated, it can either leave the active site or be bound tightly enough to bring the chemical reaction to equilibrium. In the former case activation will be observed, and in the latter, inhibition will be observed with the amount of sulfated alcohol produced determined by the relative rates of release of ROH from E–PAPS–ROH and ROSO_3^- from E–PAP– ROSO_3^- . Phenol thus appears to be a non-sticky reactant, while 2-naphthol is not.

As shown in Fig. 3, with PAP maintained at saturation, activation by phenol is only observed when pNPS and phenol are at saturation, i.e. activation results from saturation of the steady state concentration of E–PAPS with phenol. At low phenol, E–PAPS can dissociate directly to give E and PAPS with rate constant k_{15} (Reaction (2)). At saturating phenol, the expression for $\text{app } V$ contains terms for interconversion of E–PAP–pNPS to E–PAPS–pNP (not shown) and for interconversion of E–PAPS–phenol to E–PAP–phenyl sulfate (given by k'_{11} , the net rate constant for conversion of E–PAPS–phenol to E–PAP and phenyl sulfate, Reaction (2)).

(Reaction 2)

Phenol activates by binding to E–PAPS and converting it to E–PAP prior to release of PAPS. Using the method of net rate constants [19], Eq. (3) gives the expression for k'_{11} the maximum rate of formation of E–PAP from E–PAPS at saturating phenol.

$$\frac{\text{app } V}{\text{E} - \text{PAPS}} = \frac{k_{11}}{(1 + \frac{k_{11}}{k_{13}} + \frac{k_{12}}{k_{13}})} \quad (3)$$

The slow step under these conditions is likely the chemical inter-conversion step, represented by k_{11} . Since 2-naphthol does not activate, but is a good substrate for SULT1A1, it is likely that once naphthyl sulfate is formed, its release is slow, i.e. $k_{13} \ll k_{12}$.

References

1. Chapman E, Best MD, Hanson SR, Wong CH. *Chem Int Ed*. 2004; 43:3526–3548.
2. Coughtrie MW. *Pharmacogenomics J*. 2002; 2:297–308. [PubMed: 12439736]
3. Duffel MW, Marshal AD, McPhie P, Sharma V, Jakoby WB. *Drug Metab Rev*. 2001; 33:369–395. [PubMed: 11768773]
4. Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, McManus ME. *Toxicol Sci*. 2006; 90:5–22. [PubMed: 16322073]
5. Glatt H, Engelke CE, Pabel U, Teubner W, Jones AL, Coughtrie MW, Andrae U, Falany CN, Meinel W. *Toxicol Lett*. 2000; 112–113:341–348.
6. Pacifici GM. *Int J Clin Pharmacol Ther*. 2004; 42:488–495. [PubMed: 15487807]
7. Runge-Morris M, Kocarek TA. *Curr Drug Metab*. 2005; 6:299–307. [PubMed: 16101570]
8. Wang LQ, James MO. *Curr Drug Metab*. 2006; 7:83–104. [PubMed: 16454694]
9. Falany CN. *FASEB J*. 1997; 11:206–216. [PubMed: 9068609]
10. Duffel MW, Jakoby WB. *J Biol Chem*. 1981; 256:11123–11127. [PubMed: 6945304]
11. Barnett AC, Tsvetanov S, Gamage N, Martin JL, Duggleby RG, McManus ME. *J Biol Chem*. 2004; 279:18799–18805. [PubMed: 14871892]
12. Miksits M, Maier-Salamon A, Aust S, Thalhammer T, Reznicek G, Kunert O, Haslinger E, Szekeres T, Jaeger W. *Xenobiotica*. 2005; 35:1101–1119. [PubMed: 16418064]
13. Tyapochkin E, Cook PF, Chen G. *Biochemistry*. 2008; 47:11894–11899. [PubMed: 18928301]
14. Tyapochkin E, Cook PF, Chen G. *J Biol Chem*. 2009; 284:29357–29364. [PubMed: 19706609]
15. Gregory JD, Lipmann F. *J Biol Chem*. 1957; 229:1081–1090. [PubMed: 13502367]
16. Chen G, Battaglia E, Senay C, Falany CN, Radomska-Pandya A. *Protein Sci*. 1999; 8:2151–2157. [PubMed: 10548061]
17. Chen G, Rabjohn PA, York JL, Wooldridge C, Zhang D, Falany CN, Radomska-Pandya A. *Biochemistry*. 2000; 39:16000–16007. [PubMed: 11123927]
18. Falany CN, Krasnykh V, Falany JL. *J Steroid Biochem Mol Biol*. 1995; 52:529–539. [PubMed: 7779757]
19. Cleland WW. *Biochemistry*. 1975; 14:3220–3224. [PubMed: 1148201]

Fig. 1.

Initial velocity pattern obtained in the reverse reaction direction with PAP and pNPS as reactants. Rates were measured at pH 6.2 and 37 °C with PAP varied at the following concentrations: 5 μM ($\diamond$), 10 μM ($\triangle$) and 20 μM ($\circ$). Points in the primary plot are experimental, while curves are theoretical based on a fit to Eq. (2). All rates are per mg protein.

Fig. 2.

Saturation curves with pNPS as the sulfuryl donor in the reverse reaction direction. Rates were measured at pH 6.2 and 37 °C with PAP fixed at 0.1 mM and varying the concentration of pNPS as shown in the absence of alcohol (●), and in the presence of 0.1 mM 2-naphthol (Δ) or 0.1 mM phenol (○). Data were fitted using Eq. (1). All rates are per mg protein.

Fig. 3. Initial velocity pattern for activation by phenol. Rates were measured at pH 6.2 and 37 °C. The initial rate was measured as a function of pNPS with PAP fixed at 0.1 mM and phenol varied at the following phenol concentrations: 0 (●), 6.25 μM (□), 25 μM (▲), 100 μM (◇), 400 μM (△), 1.6 mM (○). Points are experimental, while the lines are based on a fit to Eq. (2). The line with closed circles is for zero phenol and was fitted separately using Eq. (1). All rates are per mg protein.

Scheme 1.

Proposed ping pong mechanism for the phenol activation of PAP sulfation by human sulfotransferase 1A1.

Table 1

Kinetic constants for SULT1A1 in the absence of added alcohol.

$V_{\max}$ (min^{-1})	0.038 ± 0.007
V/K_{PAP} ($\mu\text{M}^{-1} \text{min}^{-1}$)	0.0011 ± 0.0002
V/K_{pNPS} ($\mu\text{M}^{-1} \text{min}^{-1}$)	$(7.5 \pm 2.0) \times 10^{-5}$
K_{PAP} (μM)	34 ± 6
K_{pNPS} (mM)	5 ± 1
$K_{\text{I pNPS}}$ (mM)	25 ± 4

Table 2

Kinetic constants for activation by phenol of sulfuryl transfer from pNPS to PAP.

$V_{\max}^a$ (min ⁻¹)	0.0147 ± 0.0004
K_{pNPS}^a (μM)	23 ± 2
$V_{\max}^b$ (min ⁻¹)	0.0479 ± 0.0017
K_{pNPS}^b (μM)	60 ± 5
$K_{\text{act Ph}}$ (μM)	100 ± 10
$K_{\text{I Ph}}$ (mM)	1.5 ± 0.3

^aData obtained in the absence of phenol.^bFrom data obtained in the presence of phenol; estimates were generated using Eq. (2).