Sulfation of “Estrogenic” Alkylphenols and 17β-Estradiol by Human Platelet Phenol Sulfotransferases*

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We have investigated the ability of alkylphenols to act as substrates and/or inhibitors of phenol sulfotransferase enzymes in human platelet cytosolic fractions. Our results indicate: (i) straight chain alkylphenols do not interact with the monoamine-sulfating phenol sulfotransferase (SULT1A3); (ii) short chain 4-n-alkylphenols (C < 8) are substrates for the phenol-sulfating enzymes (SULT1A1/2), which exhibit two activity maxima against substrates with alkyl chain lengths of C1–2 and C4–5; (iii) long chain 4-n-substituted alkylphenols (C ≥ 8) are poor substrates and act as inhibitors of SULT1A1/2; (iv) human platelets contain two activities, of low and high affinity, capable of sulfating 17β-estradiol, and 4-n-nonylphenol is a partial mixed inhibitor of the low affinity form of this activity. We conclude that by acting either as substrates or inhibitors of SULT1A1/2, alkylphenols may influence the sulfation, and hence the excretion, of estrogens and other phenol sulfotransferase substrates in humans.

Endocrine disruptors are exogenous substances in the environment which can influence endocrine function in humans and other animals (1). A number of these chemicals have estrogenic activity and are thus termed “xenoestrogens.” Phytoestrogens are naturally occurring xenoestrogens produced by plants. Man-made xenoestrogens include the alkylphenols, nonylphenol and octylphenol, and bisphenol A; environmental exposure to these compounds has been reported to modify sexual development and reproductive function in amphibians (2, 3), crustacea (4, 5), and fish (6). In mammals, evidence is less clear, but there is widespread public concern that they may exert similar effects on human reproductive health and be involved in the initiation of some hormone-dependent cancers (7–14).

17β-Estradiol and alkylphenols share a common structural motif in the phenolic A ring of 17β-estradiol and the phenolic moiety of alkylphenols (Fig. 1), and it has been suggested that alkylphenols may act as endocrine disrupters by mimicking the activity of 17β-estradiol at estrogen receptors. Indeed, in a variety of cells transfected with human estrogen receptors, alkylphenols have been shown to bind weakly and provoke modest estrogenic effects (15–17). Conversely, alkylphenols have been reported to promote estrogenic signaling by inhibiting androgen receptor activation in some tissues (18, 19). However, there is also evidence that alkylphenols can disrupt endocrine-mediated events by inhibiting enzymes involved in the metabolism of sex steroids. Thus exposure of rats to octylphenol during fetal or perinatal development has been shown to decrease the expression of P450 17α-hydroxylase/C17–20 lyase (20, 21), the enzyme system responsible for the transformation of C21 steroids into C19 steroids. Hence, exposure to alkylphenols may disrupt the production of both androgens and estrogens at key times during development.

By acting as structural mimetics, alkylphenols may also disrupt the elimination of steroids from the body. Estrogenic hormones are excreted from the body following metabolic conversion to biologically less active water-soluble metabolites via cytochrome P450-mediated hydroxylation, glucuronidation, O-methylation, or sulfation (22), and many of these pathways are also utilized for the metabolism of alkylphenols in fish and mammals (23–27). Exposure of the invertebrate Daphnia magna to either nonylphenol polyethoxylate or nonylphenol has been shown to disrupt endocrine function by decreasing both the glucuronidation and sulfation of testosterone (28), but it was not demonstrated whether the alkylphenols were substrates for the D. magna testosterone sulfotransferase in this study.

Sulfation has a major role in regulating the active concentrations of a variety of biologically important molecules, including steroids, catecholamines, and peptides, and it is also important in the detoxification of many xenobiotics (29, 30). In humans, the balance of sulfation and desulfation plays an important role in modulating the activity and transport of steroid hormones: the “inactive” sulfated forms of many steroids, particularly estrone and dehydroepiandrosterone, are found in the circulation at concentrations 10–30-fold higher than the unconjugated steroids. Furthermore, sulfation appears to prolong the half-life of these compounds in the circulation. Hence steroid sulfates represent an important depot of potentially “active” steroids following desulfation by steroid sulfatases (31–33). Hydrolysis of estrone sulfate by steroid sulfatase is the major source of plasma estrogens in men and postmenopausal women, and the activity of steroid sulfatase is elevated in breast tumors (33). In fact, there is a strong inverse correlation between the level of expression of steroid sulfatase within breast tumors and disease-free survival times (34). In many breast tumors, estrogen sulfotransferase activity is much lower than in normal breast tissue, and loss of this “activating” pathway may explain why tumor cells are extremely sensitive to the mitogenic effects of estradiol and estrone sulfate (35, 36).

Sulfation reactions require PAPS1 as a sulfate donor and are catalyzed by members of the SULT family. These enzymes are widely expressed in human tissues and have been classified...

* This work was supported by a development grant from the Endocrine Modulator Steering Group of the European Chemical Industry Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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1 The abbreviations used are: PAPS, adenosine 3′-phosphate 5′-phosphosulfate; DCNP, 2,6-dichloro-4-nitrophenol; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.
Platelets were collected by centrifugation at 8,000 g for 5 min at 4 °C and then centrifuged at 100,000 g for 30 min at 4 °C. The platelets were disrupted by ultrasonication for 3 min after each burst at 4 °C and then centrifuged at 100,000 g for 60 min. The supernatant was stored in 1-ml portions at −20 °C before use.

**Measurement of Sulfotransferase Activity**—Sulfotransferase activity was measured in the platelet cytosol fractions by assessing incorporation of [35S]PAPS (mean activity 1.9 Ci/mmol) into 5′-AMP [35S]PAPS (mean activity 1.9 Ci/mmole was from NEN Life Science Products. Barium acetate, phenol, ethanol, orthophosphoric acid, zinc sulfate, and Optiphase Hisafe 3 scintillation mixture were from Fisher Scientific. 2,6-Dichloro-4-nitrophenol (DCNP) was from Fluka. Bovine hydroxide solution, Brilliant Blue G, bovine serum albumin, 2-, 3-, and 4-methylphenol, dimethyl sulfoxide, dopamine, 2-mercaptoethanol, PAPS, and TES were from Sigma. Sodium chloride and sucrose were from BDH Ltd. Alkylphenols (minimum purity 96%) were bought from the Aldrich Chemical Company with the exceptions of 3-ethylphenol (Riedel-deHae¨n, Germany); 4-n-amylyphenol, 4-n-pentlyphenol), 4-n-butylyphenol, and 4-n-heptylyphenol (Lancaster Synthesis Ltd., U. K.).

**Preparation of Platelet Cytosol Fractions from Platelet Concentrates**—Apheresis packs of platelet concentrates (stored in acid citrate buffer at 22 °C) were obtained within 5 days of donation from the National Blood Service, Birmingham, U. K. Red blood cell and lymphocyte contamination of the platelet concentrates was < 0.1% (v/v). Platelets were collected by centrifugation at 8,000 × g for 5 min at 4 °C. The pellet was washed three times with 10 mM TES-buffered saline containing 4 mM EDTA (pH 7.0 at 4 °C) and finally resuspended in one-eighth of the original pack volume of 10 mM TES storage buffer containing 0.25 M sucrose and 2 mM 2-mercaptoethanol (pH 7.0 at 37 °C). The platelets were disrupted by ultrasonication for 3 × 10-s bursts at 4 °C and then centrifuged at 100,000 × g for 60 min. The supernatant was stored in 1-ml portions at −20 °C before use.

**Sulfation of Alkylphenols and 17β-Estradiol by Human Platelets**

**EXPERIMENTAL PROCEDURES**

**Materials**—Radiolabeled 5′-[35S]PAPS (mean activity 1.9 Ci/mmole was from NEN Life Science Products. Barium acetate, phenol, ethanol, orthophosphoric acid, zinc sulfate, and Optiphase Hisafe 3 scintillation mixture were from Fisher Scientific. 2,6-Dichloro-4-nitrophenol (DCNP) was from Fluka. Bovine hydroxide solution, Brilliant Blue G, bovine serum albumin, 2-, 3-, and 4-methylphenol, dimethyl sulfoxide, dopamine, 2-mercaptoethanol, PAPS, and TES were from Sigma. Sodium chloride and sucrose were from BDH Ltd. Alkylphenols (minimum purity 96%) were bought from the Aldrich Chemical Company with the exceptions of 3-ethylphenol (Riedel-deHae¨n, Germany); 4-n-amylyphenol, 4-n-pentlyphenol), 4-n-butylyphenol, and 4-n-heptylyphenol (Lancaster Synthesis Ltd., U. K.).

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**Measurement of Sulfotransferase Activity**—Sulfotransferase activity was measured in the platelet cytosol fractions by assessing incorporation of [35S]PAPS into dopamine (SULT1A3) and 4-nitrophenol (SULT1A1/2), essentially as described in Refs. 43 and 44. These compounds are routinely used as standard substrates to assess SULT1A activities in many laboratories. To simulate in vivo conditions, TES buffer was used to adjust the pH of the assays to 7.0 at 37 °C (45). It is probable that total platelet phenol-sulfating activity is a measure of SULT1A1 and SULT1A2 activity. Hence, we have adopted the nomenclature SULT1A1/2 in the text where there may be involvement of the SULT1A2 isoform. However, the concentration of 4-nitrophenol (6.7 mM) used in most of our experiments was well below the reported Kₜₐₐₜₐ of SULT1A2 for this substrate (41); we therefore believe that under these conditions, the majority of measured phenol-sulfating phenol sulfotransferase activity was SULT1A1. Alkylphenols were dissolved and diluted in dimethyl sulfoxide, and the final concentration of the vehicle was 1% (v/v) in all incubations, including controls. Prior to use, the platelet homogenate was diluted with TES storage buffer so that the amount of PAPS consumed in the reaction was about 10% for screening enzyme activity and less than 5% for the determination of apparent kinetic constants. Platelet protein concentration was measured by the method of Bradford (46) using bovine serum albumin as standard.

**Results and Discussion**

**Alkylphenols Are Not Substrates for SULT1A3 in Platelet Cytosol**—Sulfotransferases accept a wide range of compounds as substrates, reflecting their role in xenobiotic detoxification. In view of the structural similarities between alkylphenols and known sulfotransferase substrates (Fig. 1), it seemed likely...
that these xenoestrogens may be able to influence the sulfation of other compounds by competing with multiple sulfotransferase isoforms or for the available pool of PAPS. To determine whether the alkylphenols are substrates for SULT1A3, a range of these compounds (both straight and branched chain) were incubated with platelet cytosol at a final concentration of 6.7 μM as described under "Experimental Procedures." SULT1A3 activity was determined in the presence of 6.7 μM DCNP (open symbols/bars), and SULT1A1/2 activity was calculated as total sulfation in the absence of DCNP less that in the presence of the inhibitor (closed symbols/bars). Sulfation of the alkylphenols is expressed relative to sulfation of appropriate standard substrates (4-nitrophenol for SULT1A1/2 and dopamine for SULT1A3) in parallel incubations. Data in panel a are means ± S.E. for 9–12 determinations from three separate platelet preparations. Data in panel b are means ± S.E. from triplicate determinations from a single platelet preparation.

Fig. 2. Short chain alkylphenols are substrates for SULT1A1/2 but not SULT1A3 in human platelet cytosol. Human platelet cytosol fractions were incubated with alkylphenols or standard substrates at a final concentration of 6.7 μM as described under "Experimental Procedures." SULT1A3 activity was determined in the presence of 6.7 μM DCNP (open symbols/bars), and SULT1A1/2 activity was calculated as total sulfation in the absence of DCNP less that in the presence of the inhibitor (closed symbols/bars). Sulfation of the alkylphenols is expressed relative to sulfation of appropriate standard substrates (4-nitrophenol for SULT1A1/2 and dopamine for SULT1A3) in parallel incubations. Data in panel a are means ± S.E. for 9–12 determinations from three separate platelet preparations. Data in panel b are means ± S.E. from triplicate determinations from a single platelet preparation.
mine sulfate is likely to be the major product in these coincubations.

Alkylphenols with Alkyl Chains of C < 8 Are Substrates for SULT1A1/2 in Platelet Cytosol—Phenol and a series of 4-n-alkylphenols with straight alkyl chains increasing from 1 to 9 carbons in length were tested as substrates for SULT1A1/2. Fig. 2a shows that their ability to act as substrates for SULT1A1/2 was inversely related to the alkyl chain length and hence the hydrophobicity of the compounds. At a concentration of 6.7 μM 4-n-propylphenol with 6.7 μM dopamine + DCNP (SULT1A3 activity, □) or 6.7 μM 4-nitrophenol (± DCNP) was determined as described under “Experimental Procedures.” SULT1A1/2 activity was calculated as described for Fig. 2 (●). Data are means ± S.E. of duplicate determinations from three experiments using two separate platelet preparations.

Fig. 3. Long chain (C > 7) 4-n-alkylphenols (panel a) and selected branched chain alkylphenols (panel b) inhibit SULT1A1/2-mediated sulfation of 4-nitrophenol but have no effect on SULT1A3-mediated sulfation of dopamine in human platelet cytosol. Total sulfate incorporated in coincubations of 6.7 μM 4-n-alkylphenol with 6.7 μM dopamine + DCNP (SULT1A3 activity, □) or 6.7 μM 4-nitrophenol (± DCNP) was determined as described under “Experimental Procedures.” SULT1A1/2 activity was calculated as described for Fig. 2 (●). Data are means ± S.E. of duplicate determinations from three experiments using two separate platelet preparations.

Fig. 2b also shows that at a concentration of 6.7 μM, 2-methylphenol, 2-ethylphenol, 2-n-propylphenol, 3-methylphenol, and 3-ethylphenol can be sulfated by human platelet SULT1A1/2. Unfortunately, alkylphenols with longer alkyl chains on the 2- or 3- positions were not commercially available so we were unable to investigate whether these compounds were SULT1A1/2 substrates or inhibitors. We also tested a limited number of branched chain alkylphenols as substrates for SULT1A1/2. Fig. 2b shows that their ability to act as substrates decreased as the bulk of the alkyl side chain increased. This is most clearly shown by the 4-butylphenol series, where the rates of sulfation of 6.7 μM 4-n-butylphenol, 4-sec-butylphenol, and 4-tert-butylphenol were ~90%, ~65%, and ~28%, respectively, of that of 6.7 μM 4-nitrophenol.

To examine these phenomena further we compared the kinetic properties of the sulfation of 4-nitrophenol with those of a series of 4-substituted straight chain alkylphenols. The results (Fig. 4) show that the kinetic profiles for the sulfation of both 4-nitrophenol and two short chain 4-alkylphenols (4-methylphenol and 4-n-amylphenol) were essentially identical: as the substrate concentration was increased, the initial rates rose to a peak value before falling to a lower plateau. All experiments were performed at a saturating concentration of PAPS, so these results cannot be explained by depletion of this vital cosubstrate. Hence it is likely that binding of the substrate affects the initial rate of catalysis. A similar effect has recently been reported with SULT1A1/2 (48) and recombinant human estrone.
gen sulfotransferase (SULT1E) (49). Zhang and co-workers (49) have examined this phenomenon in some detail using recombinant human SULT1E and have shown that two estrogen molecules bind per SULT1E monomer. They suggest that the partial inhibition occurs after the binding of estrogen to an allosteric regulatory site. We suggest that 4-nitrophenol and short chain 4-alklyphenols may also interact with two common sites on each SULT1A1/2 monomer, thus influencing the initial rate of catalysis.

The kinetic data shown in Fig. 4 were fitted to an equation (Equation 1) describing the behavior of an enzyme system in which substrate molecules bind first to the active site to generate product and second to an allosteric site that inhibits catalysis (50).

\[
v = \frac{V_{\text{max}}(1 + (V_{\text{max}} \times [S]/V_{\text{max}} \times K_v))}{1 + K_v[S] + [S]/K_i}
\]  

(Eq. 1)

The kinetic constants \( V_{\text{max}} \) and \( K_m \) were obtained from initial rate studies using low substrate concentrations at which inhibitory effects were negligible. For each substrate, \( V_{\text{min}} \) was set at a value slightly below the rate of product accumulation during the plateau phase (49, 50). The equation assumes that the PAPS concentration used in the assay was saturating and that substrate binding was at equilibrium. The best fit \( K_v \) values for 4-nitrophenol, 4-ethylphenol, and 4-4-amylyphenol were 5.2 ± 0.8, 12.3 ± 3.2, and 15.1 ± 0.32 \( \mu \)M, respectively. Hence the kinetic behavior of 4-nitrophenol and the short chain 4-alklyphenols (Fig. 4) is consistent with the possibility that, as has been suggested for the interaction of estradiol and SULT1E (49), these compounds act both as substrates for SULT1A1/2 and as an allosteric site that inhibits catalysis.

The kinetic parameters for a series of straight chain alklyphenols were determined at low concentrations so that the inhibitory influences of the allosteric regulatory site were negligible (Table I). Although the \( K_m \) values obtained will be useful for comparing the substrate preferences of SULT1A1/2 for the different alklyphenols, they do not represent a direct measure of substrate affinity. Rather, they will be a compound reflection of several processes in the catalytic cycle and will be highly dependent on the rate constants for catalysis itself and for product release. Therefore, a better indicator of substrate preference is the ratio \( V_{\text{max}}/K_m \) which is known as the specificity constant (51). Because the absolute magnitude of \( V_{\text{max}} \) for each substrate will vary slightly among platelet preparations, the ratio of the specificity constants for the alklyphenols relative to that of 4-nitrophenol measured in the same experiment can be used to determine the substrate preferences of human platelet SULT1A1/2 (Table I). Of the 4-substituted phenols, 4-methylphenol was the optimal SULT1A1/2 substrate, having a specificity constant slightly greater than that of 4-nitrophenol (as did 2-methylphenol and 2-ethylphenol), which is in agreement with the \( \delta \) incorporation experiments shown in Fig. 2. 4-n-Propylphenol appeared to be the least favorable substrate, having a specificity constant just 5% of that of 4-nitrophenol. As the alkyl chain length increased there was a slight recovery in activity relative to 4-nitrophenol; however, the specificity constants for 4-n-butylphenol and 4-n-amylyphenol were still only 30% that of 4-nitrophenol. Because longer chain 4-n-alklyphenols were such poor substrates, it was not possible to obtain accurate specificity constants for these compounds.

4-Ethylphenol was a moderately good substrate for SULT1A1/2; the results in Table I and Fig. 3b suggest that it may compete with other substrates for this enzyme. This is of particular interest in view of recent evidence that 4-ethylphenol is a major metabolite of the dietary phytoestrogen genistein in rats and humans (52). The daily exposure of humans to genistein may be several hundredfold greater than that to environmental alklyphenols (53), but it remains to be determined if dietary phytoestrogens can generate sufficient 4-ethylphenol to compromise SULT1A1/2 activity in target tissues.

The conformation of the 4-alklyphenol chain also plays an important role in determining whether the alklyphenols were SULT1A1/2 substrates. This is best illustrated with the 4-butylphenol series (Table II); the specificity constant of 4-n-butylphenol was approximately 27% of that of 4-nitrophenol, whereas the relative specificity constants for 4-sec-butylphenol and 4-tert-butylphenol were -3% and -1%, respectively. For each of these compounds the \( V_{\text{max}} \) values were similar, but \( K_m \) increased with increasing chain branching. Therefore, introduction of bulky side groups onto the 4-position may affect substrate binding or orientation within the active site.

We have been able to test the effects of changing the position of the alkyl chain relative to the phenolic hydroxyl group for both methylphenol and ethylphenol. Both of these compounds were substrates for SULT1A1/2 irrespective of the position of the alkyl group on the phenol ring (Fig. 2b). However, the kinetic data in Table I indicate that for both methyl- and ethylphenol, the 2-substituted compounds were the preferred substrates. It has been noted that the catecholestrogens, and 2-hydroxyestradiol in particular, are much better SULT1A1/2 substrates than is 17β-estradiol (42). Perhaps having a small...
Sulfation of Alkylphenols and 17β-Estradiol by Human Platelets

Enzyme activities were determined in duplicate samples from platelet cytosol preparations from three separate individuals as described under “Experimental Procedures.” $K_m$ and $V_{\text{max}}$ values were calculated using the EnzFitter software program and are given as means ± S.E. For further explanation, see “Results and Discussion.”

### TABLE I

| Compound                  | $K_m$ (μM) | $V_{\text{max}}$ (pmol/min) | Specificity constant $V_{\text{max}}/K_m$ | Specificity constant relative to 4-nitrophenol |
|---------------------------|------------|------------------------------|--------------------------------------------|-----------------------------------------------|
| Phenol                    | $3.83 ± 0.25$ | $1.85 ± 0.05$                | $0.48$                                      | $0.12$                                        |
| 2-Methylphenol            | $0.15 ± 0.02$ | $1.38 ± 0.03$                | $9.20$                                      | $2.45$                                        |
| 3-Methylphenol            | $1.53 ± 0.12$ | $1.56 ± 0.04$                | $1.02$                                      | $0.27$                                        |
| 4-Methylphenol            | $0.50 ± 0.03$ | $1.39 ± 0.02$                | $4.83$                                      | $1.23$                                        |
| 2-Ethylphenol             | $0.05 ± 0.02$ | $1.01 ± 0.03$                | $20.2$                                     | $5.40$                                        |
| 3-Ethylphenol             | $0.78 ± 0.10$ | $1.59 ± 0.04$                | $2.03$                                      | $0.54$                                        |
| 4-Ethylphenol             | $1.16 ± 0.07$ | $1.73 ± 0.03$                | $1.52$                                      | $0.41$                                        |
| 4-n-Propylphenol          | $6.22 ± 0.53$ | $1.33 ± 0.05$                | $0.21$                                      | $0.06$                                        |
| 4-n-Butylphenol           | $1.06 ± 0.10$ | $1.06 ± 0.08$                | $1.00$                                      | $0.27$                                        |
| 4-n-Amylphenol            | $0.75 ± 0.08$ | $1.06 ± 0.03$                | $1.41$                                      | $0.38$                                        |
| 4-Nitrophenol             | $0.31 ± 0.05$ | $1.16 ± 0.06$                | $3.74$                                      | $1.00$                                        |

### TABLE II

**Effect of chain conformation on the kinetic parameters of SULT1A1/2-mediated sulfation of 4-butylphenol compared with 4-nitrophenol**

Enzyme activities were determined in duplicate samples from platelet cytosol preparations from three separate individuals as described under “Experimental Procedures.” $K_m$ and $V_{\text{max}}$ values were calculated using the EnzFitter software program and are given as means ± S.E. For further explanation, “Results and Discussion.”

| Compound                  | $K_m$ (μM) | $V_{\text{max}}$ (pmol/min) | Specificity constant $V_{\text{max}}/K_m$ | Specificity constant relative to 4-nitrophenol |
|---------------------------|------------|------------------------------|--------------------------------------------|-----------------------------------------------|
| 4-n-butylphenol           | $1.06 ± 0.10$ | $1.06 ± 0.08$                | $1.00$                                      | $0.27$                                        |
| 4-sec-butylphenol         | $9.60 ± 0.95$ | $1.15 ± 0.08$                | $0.12$                                      | $0.03$                                        |
| 4-tert-butylphenol        | $31.5 ± 2.72$ | $0.90 ± 0.03$                | $0.03$                                      | $0.01$                                        |

methyl- or ethyl- group adjacent to the phenolic hydroxyl group may serve as a structural mimetic of the catecholestrogen motif.

**Long Straight Chain and Branched, 2-Substituted, Short Chain Alkylphenols Inhibit Human Platelet SULT1A1/2 Activity**—Longer straight chain alkylphenols such as 4-n-octylphenol and 4-n-nonylphenol were very poor substrates for either SULT1A1 or SULT1A2 because they were sulfated at < 10% of the rate of 4-nitrophenol, even at concentrations up to 100 μM. However, 6.7 μM 4-n-heptyl-, 4-n-octyl-, and 4-n-nononylphenol significantly reduced (by 30%, p < 0.005; 24%, p < 0.005; and 17%, p = 0.05, respectively) the accumulation of sulfated products generated in coincubations with 6.7 μM 4-nitrophenol (these are likely to be almost exclusively 4-nitrophenol sulfate, Fig. 3a). When these coincubation experiments were repeated with 6.7 μM 4-nitrophenol and 100 μM 4-n-heptyl-, 4-n-octyl-, or 4-n-nononylphenol, the accumulation of sulfated products was decreased by 57%, 82%, and 86%, respectively (p < 0.001 in each case, results not shown). Hence, although 4-n-nononylphenol is not a significant substrate for SULT1A1/2 (even at 100 μM, results not shown), it can inhibit sulfation of 4-nitrophenol, perhaps via the putative allosteric site.

In most cases, coincubating 6.7 μM branched chain alkylphenols with 6.7 μM 4-nitrophenol did not significantly alter total $^{35}$S incorporation into sulfated products, regardless of the position of substitution. The highly branched 4-tert-octylphenol had no discernible effect upon total sulfation in the presence of either standard substrate, even at a concentration of 100 μM. However, coincubation of 4-nitrophenol with either 2-sec-propylphenol or 2-sec-butylphenol, both poor substrates for SULT1A1/2, caused a significant (Fig. 3b, ~ 70%, p < 0.001) inhibition of the accumulation of sulfated products. The structure of 2-sec-propylphenol is similar to that of the aspirin metabolite salicylic acid (2-hydroxybenzoic acid), which we have previously shown to be a highly selective inhibitor of 4-nitrophenol sulfation (54). Coincubation of 4-nitrophenol with 2-ethylphenol, 2-n-propylphenol, or 2-tert-butylphenol caused much less inhibition of total sulfation (~ 10–20%), suggesting that although a branched chain is important for inhibition, it may also be necessary for the alkylphenol to adopt a planar configuration to interact with the enzyme(s).

**17β-Estradiol Is Sulfated in Human Platelet Cytosol by Two Separate Sulfotransferase Activities That Are Inhibited by 4-n-Nonylphenol**—It has been reported that human platelets do not express estrogen sulfotransferase activity (55). However, because 17β-estradiol has been shown to be a SULT1A1/2 substrate in human liver, breast cancer cells, and fetal lung extracts ($K_{\text{m}}$ ~ 2–5 μM (39–42, 56), it seemed likely that it may be sulfated by a similar route in platelets.

Fig. 5a shows that 17β-estradiol (0.1–200 μM) was sulfated in human platelet cytosol in a concentration-dependent fashion. The pattern of 17β-estradiol sulfation was different from that
seen with 4-nitrophenol and the short chain 4-alkylphenols. Thus the rate of sulfate incorporation increased with substrate concentration between 0.1 and 30 μM; above 30 μM the rate of sulfate incorporation reached a plateau that was approximately 20% of the maximal rate of 4-nitrophenol sulfation. We interpret these data to indicate that 17β-estradiol is not able to influence the catalytic function of the enzyme by binding to the putative allosteric site. However, 17β-estradiol sulfation does not appear to follow strict Michaelis-Menten kinetics. An Eadie-Hofstee transformation of the kinetic data in Fig. 5 shows that human platelets contain two enzyme species capable of sulfating 17β-estradiol. One species had a relatively low affinity ($K_m = 2.4 \pm 0.15 \mu M$) of the same order of magnitude as the reported $K_m$ of human SULT1A1/2 for 17β-estradiol (39–42). The second species had a much higher affinity for 17β-estradiol ($K_m = 0.043 \pm 0.01 \mu M$); this value is approximately an order of magnitude greater than the reported $K_m$ (5 nM) of recombinant human estrogen sulfotransferase (49). Because the platelet preparations are essentially devoid of contamination with other blood cells it is likely that this “high” affinity activity is a true platelet protein. The identity of this enzyme is unknown at present, and its relatively low activity precluded further investigation in the present study.

We have examined the effects of 4-n-nonylphenol (1–20 μM) on the sulfation of 17β-estradiol in human platelets by the “low” affinity activity, which we presume to be SULT1A1/2. Despite its low affinity for 17β-estradiol, this activity may be of significance in estrogen-dependent mammary tumors, where the local concentration of the hormone has been reported to approach 1 μM in some cases (57). The transformations of the kinetic data describing the effects of 4-n-nonylphenol on estradiol sulfation by SULT1A1/2 are shown in Fig. 6; they demonstrate that 4-n-nonylphenol acts as a partial mixed inhibitor of SULT1A1/2-mediated estradiol sulfation. This behavior is similar to that of several other known inhibitors of SULT1A1/2 activity including vanillin (58) and quercetin (59). We suggest that the most likely explanation of this complex enzymatic behavior is that 4-n-nonylphenol can bind to free SULT1A1/2 yielding an enzyme-inhibitor complex (dissociation constant $K_i = 2.8 \mu M$) and also bind to the SULT1A1/2–17β-estradiol complex giving an unreactive enzyme-substrate/inhibitor complex (dissociation $K_i = 5.4 \mu M$). Thus 4-n-nonylphenol may both competitively antagonize the binding of 17β-estradiol to the active site and also interact noncompetitively with the allosteric regulatory site to decrease sulfation. Further work using purified recombinant SULT1A1/2 will be needed to establish the exact nature of the inhibition of 17β-estradiol sulfation by 4-n-nonylphenol and other alkylphenols.

Agents that inhibit SULT1A1/2 activity, such as 4-n-nonyl-
Sulfation of Alkylphenols and 17β-Estradiol by Human Platelets

phenol, may be able to increase local estradiol concentrations in those tissues in which SULT1A1/2 is the only route of steroid sulfation. This may be the case in hormone-dependent tumors of the breast. For example, the MCF-7 hormone-dependent breast cancer cell line has a very active estrone sulfatase pathway (34–36), but no significant cytosolic estrogen or hydroxysteroid sulfotransferase activity can be detected (39–41), and SULT1A1/2 is the only relevant pathway for steroid sulfation (42). If a similar situation exists in human mammary tumors, alkylphenols may be able to interfere with the “detoxification” of high levels of 17β-estradiol in these tissues. This may be an important clinical factor because reduced estrogen and dehydroepiandrosterone sulfation in tumor homogenates from breast cancer patients is associated with a poor responsiveness to endocrine or ablative therapy and a grim clinical prognosis (60, 61).

In conclusion, it appears that a number of alkylphenols may be sulfated by SULT1A1/2 and/or act as inhibitors of these enzymes. The sulfation of short chain alkylphenols probably represents a detoxification pathway, but in organisms subject to high levels of exposure to these compounds, extensive alkylphenol sulfation may reduce the sulfation and elimination of endogenous substrates such as estrogens by competing for the available pool of PAPS. Longer chain and branched chain alkylphenols, which are now widespread in the environment, are potent inhibitors of SULT1A1/2 and would be expected to reduce the sulfation of endogenous estrogens, especially in tissues such as mammary tumors where this pathway represents a major route of steroid sulfation and elimination (42). It is also possible that alkylphenol sulfates may themselves present a toxic threat to the organism by influencing the activity of a variety of endogenous sulfatases (62). The ability of alkylphenols to reduce estrogen sulfation may lead to a localized accumulation of free estrogens in tissues, thereby provoking the “environmental estrogenic” effects with which these compounds have become associated.

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