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INHIBITION OF PHENOL SULFOTRANSFERASE BY PYRIDOXAL PHOSPHATE

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Abstract—The biologically abundant cofactor, pyridoxal-5-phosphate (PLP), is a potent inhibitor of bovine phenol (aryl) sulfotransferase (PST). Preincubation of purified enzyme with as little as 1 μM PLP decreased PST activity by 50%. Excess 2-naphthol protected PST from inactivation by PLP, whereas 2-naphthyl sulfate and PAPS were not protective. Although PLP inhibition was apparently competitive with 2-naphthol, a steady-state kinetic K_i value could not be measured due to non-linear Lineweaver–Burk plots in the presence of the inhibitor. Kinetic progress curves revealed that this was due to progressive loss of activity during catalysis. The kinetics of inactivation of PST by PLP were pseudo-first-order and exhibited saturation. The derived K_I value for the binding of PLP to PST in the initial reversible step was 23 μM, with a maximal rate of inactivation of 0.077 min^{-1}. Absorbance spectra of the PST/PLP complex indicated the formation of a Schiff base conjugate, and this is consistent with decreased electrophoretic mobility of the protein–PLP adduct in the presence of dodecyl sulfate only after reduction with borohydride. These results point to the possible regulation of an important detoxification enzyme by a ubiquitous cofactor.

Key words: phenol; sulfotransferase; pyridoxal phosphate; inhibition; bovine; lung

Metabolism of xenobiotics occurs by means of phase I or activating enzymes, such as the various cytochromes P450 [1, 2], and by phase II or conjugative enzymes, such as the glutathione transferases. Another family of phase II enzymes, the sulfotransferases, catalyzes the transfer of a sulfuryl group, SO_3, from the donor substrate PAPS† to acceptor substrates. The phenol or aryl sulfotransferases (PSTs, EC 2.8.2.1) are a subfamily with differential specificities for varied hydroxylated aromatic compounds and form the corresponding sulfate esters [3, 4].

Although an accurate classification of the sulfotransferases will emerge with the acquisition of precise molecular sequence information [5–9], the earlier classifications of the phenol sulfotransferases based on both substrate specificities and physical characteristics still provide a useful framework. For example, humans express two PSTs: the M-PST rapidly sulfates micromolar concentrations of catecholamines [10], whereas the P-PST enzyme prefers simple phenols [11]. The M-PST is sometimes referred to as a thermolabile enzyme, or TL-PST, due to its sensitivity to heat in comparison to the P-PST, which is often called thermostable, or TS-PST [12, 13]. In this context, bovine PST is reportedly thermostable and unable to sulfate catecholamines [14]. Therefore, if extrapolations are to be made based on this current work, they may be most valid for the human P-PST.

Early investigations of PSTs indicated the existence of an active site nucleophile that, when modified with thiol-directed reagents such as *N*-ethylmaleimide (NEM), caused inhibition of enzyme activity [15]. Estimates of stoichiometry indicated binding of two molar equivalents of NEM [16], and inclusion of either PAPS or *p*-nitrophenol reduced binding [16] and protected against enzyme inactivation [11, 16]. Thus, two active site cysteinyl residues have been implicated, one in each of the substrate binding domains.

Additional phenol sulfotransferase active site nucleophiles have been reported. Reaction of aldehyde and ketone organic reagents with preferential reactivity toward arginyl residues suggested the presence of two such groups, one in each of the two substrate binding sites [16]. Other studies have revealed that synthetic nucleotides with aldehyde moieties derived from the ribose ring are also active-site-directed inhibitors, and protection against inactivation by excess PAPS indicated that a reactive nucleophile therefore resides within the "donor" or PAPS binding domain [16]. In summary, as many as four PST active-site nucleophiles can be inferred from the existing data.

Considerable emphasis has been placed both on delineating the steady-state kinetic mechanisms of phenol sulfotransferases [17–19] and on determining the substrate specificities for phenolic acceptor substrates by quantitative structure–activity relationships [12, 19]. These data were used in the successful design of a phenolic azido photoaffinity label [13]. Not easily predicted by such studies, however, are

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† Abbreviations: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PST, phenol sulfotransferase; and PLP, pyridoxal-5-phosphate.

reports demonstrating the inhibition of a phenol sulfotransferase by aryl carboxylic acids [20]. Taking these results together, we have postulated that aromatic compounds containing both a negatively charged side group and an electrophilic aldehyde moiety could be especially effective phenol sulfotransferase inhibitors. Thus, PLP was chosen for the initial investigation, meeting these structural requirements plus being an abundant and likely relevant natural product. Here we report that PLP is indeed a potent inhibitor of bovine phenol sulfotransferase, and that it binds to the enzyme as a Schiff base probably within the acceptor domain.

MATERIALS AND METHODS

Reagents. Unlabeled PAPS and [^{35}S]PAPS (2 Ci/mmol) were from Pharmacia (Piscataway, NJ) and New England Nuclear (Boston, MA), respectively. 2-Naphthol was from Fluka (Ronkonkoma, NY), whereas 2-naphthyl sulfate was from Research Organics (Cleveland, OH). Pyridoxal phosphate was acquired from both Sigma (St. Louis, MO) and ICN (Costa Mesa, CA), and these reagents produced similar results. Pyridoxal, pyridoxamine, pyridoxamine 5-phosphate, and 3-hydroxypyridine were from Sigma. Other reagents were of the highest purities available.

Enzyme analyses. Phenol sulfotransferase activity was measured at 23° using a modified [21] radioisotopic procedure in which ^{35}S-labeled naphthyl sulfate is separated from [^{35}S]PAPS by ascending thin-layer chromatography [22, 23]. The assay buffer consisted of 50 mM succinate, 50 mM phosphate, 2 mM 2-mercaptoethanol, pH 5.5 [24]. One enzyme unit catalyzes the formation of 1 nmol naphthyl sulfate/min. Concentrations of 2-naphthol and total PAPS are indicated in the figure legends. For single time-point measurements, the extent of transfer of sulfate from PAPS to labeled product was 10% or less.

Phenol sulfotransferase preparation. Phenol sulfotransferase was prepared from bovine lung parenchyma using methods common to the purification of these enzymes from other sources [13, 25, 26]. Fresh lung tissue was homogenized, by a Waring blender, into 20 mM Tris–HCl/5 mM 2-mercaptoethanol/10% (w/v) sucrose/1 mM EDTA/pH 7.5 (Buffer B) plus 1 mM phenylmethylsulfonyl fluoride (PMSF), followed by a 45,000 *g* centrifugation to prepare clear crude cytosolic extracts. These extracts were then fractionated by ammonium sulfate precipitation; the centrifugal pellets obtained at 30–55% saturation were dissolved in, and exhaustively dialyzed against, Buffer B. After centrifugation to remove precipitated protein, the dialysates were applied to DEAE cellulose (DE-52, Whatman, 2.5 × 45 cm) equilibrated in Buffer B. Following a 500–1000 mL wash in this buffer, a linear 1.8 L gradient of NaCl to 0.4 M was applied. Fractions containing the greatest PST enzyme activity eluting midway through the gradient were pooled and applied directly to a column (2.5 × 10 cm) of Reactive green agarose (Sigma). This column was then washed with Buffer B containing 0.4 M NaCl until the 280 nm absorbance reached baseline, at which point PST activity was eluted by application of Buffer B containing 2 M NaCl. This material was concentrated by the addition of solid ammonium sulfate to 60% saturation and then centrifuged; the pelleted protein was dissolved in 4–5 mL of 20 mM HEPES/20% sucrose/5 mM β-mercaptoethanol/1 mM EDTA/pH 7.4 (Buffer A). This was directly passed over AcA 34 (Spectrum, 2.5 × 48 cm) in Buffer A, and fractions containing the greatest PST activity were pooled and concentrated using Centricon ultrafiltrators (Amicon), to 1–2 mL. Finally, enzyme was applied to 1-mL bed volumes of ADP: agarose (Sigma) in Buffer A, washed with 10–15 mL Buffer A, and PST was eluted using 5 mL of Buffer A containing 50 μM PAPS. Analysis of purity by SDS–PAGE [27] revealed the anticipated [28] 32-kDa subunits with a calculated level of impurities of less than 5%. The purest fractions had final specific activities of 1500–1700 nmol/mg protein/min (U/mg) when assayed using 100 μM 2-naphthol, 125 μM total PAPS, at pH 5.5, which is the optimal assay pH under these conditions (see below and Ref. 24). This specific activity is comparable to the greatest values reported for other phenol sulfotransferases [4, 11, 15, 29, 30], although it is approximately 500-fold greater than the previously reported value for the bovine lung enzyme [28]. Purified enzyme was stored at 5° in 20 mM HEPES/20% sucrose/5 mM β-mercaptoethanol/1 mM EDTA/pH 7.4 (Buffer A). The results reported here were repeated with two and sometimes three different enzyme preparations.

Tests of pyridine-based compounds as sulfotransferase substrates. The various compounds used in this investigation (see Fig. 1) were tested as sulfate acceptors as follows. For qualitative assessment of all compounds, reactions (10 μL) containing ±0.3 U phenol sulfotransferase, 0.2 μCi [^{35}S]PAPS (no carrier PAPS), and ±100 μM concentration of compounds I–VI (see Fig. 1) were incubated for 1 hr at 23°. The samples were subjected to ascending thin-layer chromatography, air dried, and subsequently exposed for 24–48 hr to Kodak XAR-5 film. For quantitative assessment of 3-hydroxypyridine sulfation compared with 2-naphthol, reaction progress curves were measured in the presence of excess PAPS.

Inhibition of phenol sulfotransferase by pyridoxal-5-phosphate. For the initial studies of PLP concentration-dependent PST inhibition, enzyme plus PLP and/or PAPS, 2-naphthol, or 2-naphthyl sulfate were preincubated for 30 min in Buffer A (above) prior to 10-fold dilution into the assay. For measurements of the effects of PLP on PST catalysis without preincubation, aliquots of enzyme in Buffer A were combined with solutions containing PAPS, 2-naphthol, and PLP in the assay buffer to achieve final concentrations as described above and in the figure legends. For measurements of pseudo-first-order rates of inactivation, aliquots from incubations of enzyme plus varied concentrations of PLP in Buffer A were diluted (40%) into assay buffer (see above) containing both PAPS and 2-naphthol (100 μM). The subsequent assay incubation times were only 1.5 min in order to minimize introducing

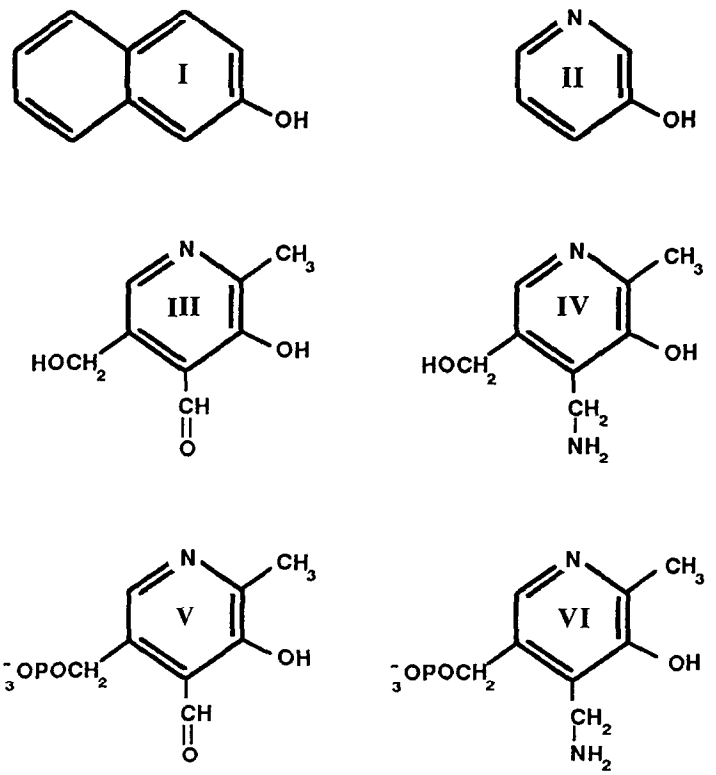

Fig. 1. Chemical structures of substrates and inhibitors. 2-Naphthol (I) was the routine acceptor substrate for PST assays. 3-Hydroxypyridine (II) was used to determine if this base structure could be sulfated by PST. Pyridoxal (III), pyridoxamine (IV), pyridoxal-5-phosphate (PLP,V), and pyridoxamine-5-phosphate (VI) are members of the vitamin B_6 family.

error due to continued inactivation during the assay. All incubations were conducted at 23°.

Spectral characterization of the PST:PLP complex. Purified enzyme was incubated ±equimolar PLP in Buffer A for 30–40 min prior to measurement of absorption spectra using a Beckman DU-62 spectrophotometer. Excess PLP was not used for these experiments in order to observe the possible spectral shifts of PLP upon binding with the enzyme.

Electrophoretic characterization of the PST:PLP interaction. Purified enzyme in Buffer A was preincubated ± excess but sufficient PLP (330 μM) to ensure 80–90% inhibition for a 30-min exposure. These initial incubations then received ± excess (1.25 mM) sodium borohydride, freshly prepared as an 8x concentrate in water. After a further 30 min at room temperature, samples were heat-denatured at 95° for 5 min in the presence of 1% (w/v) sodium dodecyl sulfate plus 5% (v/v) 2-mercaptoethanol. Polyacrylamide gels were prepared and handled according to a modification [31] of the Laemmli method [27] and subsequently fixed and stained with Coomassie R-250.

RESULTS

The chemical structures of the substrates and inhibitors used in this investigation are shown in Fig. 1. 2-Naphthol (I) was the routine acceptor substrate for the measurement of phenol sulfotransferase activities. 3-Hydroxypyridine (II) was a test compound used to assess the ability of this class of structures to be sulfated by the bovine enzyme. Compounds III–VI are various members of the vitamin B_6 family, with pyridoxal-5-phosphate (V) having the greatest importance for the purposes of this investigation.

The ability of phenol sulfotransferase to use various pyridine ring-based structures as acceptor substrates was tested (Fig. 2). Conditions of excess enzyme, excess time, and absence of non-radioisotopic PAPS were used, in order to maximize the extent of sulfate transfer and product detection. In the routine ascending thin-layer chromatographic system, unreacted [^{35}S]PAPS remained near the origin (Fig. 2, lane 8). Complete conversion of the label to naphthyl sulfate was observed upon inclusion of 2-naphthol (Fig. 2, lane 1). In contrast, incomplete conversion occurred in the presence of 3-hydroxypyridine (Fig. 2, lane 2), and no obvious conversions were observed with the members of the vitamin B_6 family (Fig. 2, lanes 3–6). A small amount of apparent product was observed in the presence of enzyme only (Fig. 2, lane 7), which accounts for the trace conversion in the B_6 reactions; this may be due to sulfation of a buffer component or an impurity therein, since omission of enzyme showed no product formation (Fig. 2, lane 8). It is important to note

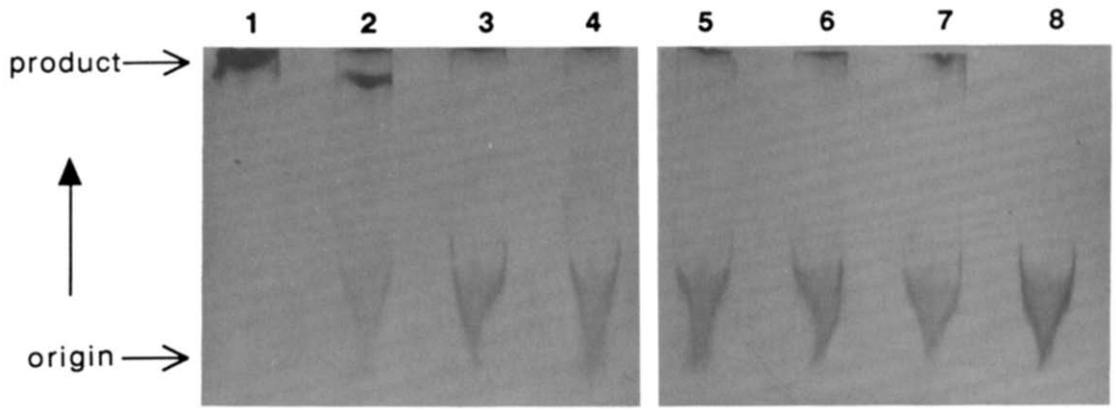

Fig. 2. Ascending thin-layer chromatographic analysis of reaction products produced by phenol sulfotransferase. Incubations (1 hr) included 0.3 U enzyme (lanes 1–7) at pH 5.5 and 23°, 0.2 μCi [^{35}S]-PAPS, and a 100 μM concentration of the following compounds in a final volume of 10 μL: 2-naphthol (lane 1), 3-hydroxypyridine (lane 2), pyridoxal-5-phosphate (lane 3), pyridoxal (lane 4), pyridoxamine (lane 5), and pyridoxamine-5-phosphate (lane 6). Lane 7 included enzyme but no acceptor substrate, and lane 8 is representative of the controls lacking enzyme ± the various compounds.

that 3-hydroxypyridine sulfate did not migrate with the solvent front (Fig. 2, lane 2), and this suggests the possibility that the hypothetical vitamin B_6 derivatives may have migrated anomalously. Therefore, the migration of compounds III–VI (Fig. 1) was measured and found to have R_f values of 0.80 ± 0.05 (III), 0.76 ± 0.07 (IV), 0.42 ± 0.07 (V) and 0.41 ± 0.07 (VI). Thus, the presence of the negatively charged phosphate group is correlated with a reduction in migration, and such an effect would also be predicted for the sulfated products of pyridoxal (III) and pyridoxamine (IV). There was no evidence, however, for the formation of such products with an anticipated mobility (Fig. 2, lanes 4 and 5).

A quantitative comparison of the sulfation of 2-naphthol with 3-hydroxypyridine (II) was made (Fig. 3). The initial specific velocities indicated that the pyridine-based substrate was sulfated at less than 1% the rate of 2-naphthol. It seems likely that this effect may be due to the ring nitrogen and electronic effects on the hydroxyl group. The apparent lack of sulfation of the more complex pyridoxine-base compounds (III–VI) may be due to such an effect, in addition to possible steric hindrance by the ring substituents adjacent to the hydroxyl group.

To determine if phenol sulfotransferase could be inhibited by PLP (Fig. 1, V), purified enzyme was preincubated with varied concentrations of PLP, and the resulting activities were measured (Fig. 4). Fifty percent inhibition was achieved with as little as 1 μM PLP. Similar concentration dependency was observed with two separate enzyme preparations and different PLP sources. In addition, omission of the potential competing nucleophile 2-mercaptoethanol [32] from the preincubation and assay buffers did not alter appreciably the PLP concentration dependence of inhibition (data not shown).

Other pyridoxine-related compounds (Fig. 1) were tested for inhibition of phenol sulfotransferase. After

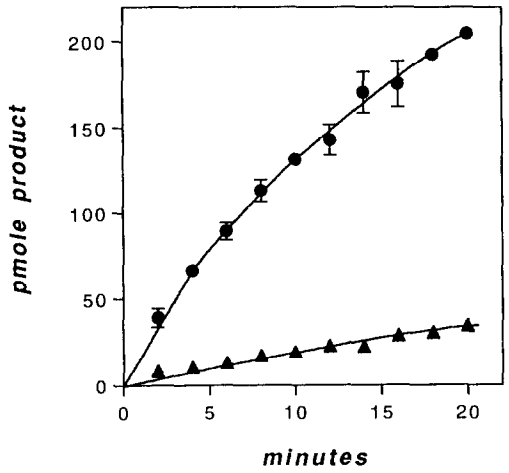

Fig. 3. Reaction progress curves for the sulfation of 2-naphthol and 3-hydroxypyridine. Each 300-μL incubation at pH 5.5 and 23° included 124 μM total PAPS, and a 100 μM concentration of either 2-naphthol (●, 0.6 U enzyme) or 3-hydroxypyridine (▲, 9 U enzyme). At the indicated times, 10-μL aliquots were quenched with acetic acid, and product was quantified after thin-layer chromatography. Each point is the average of triplicate measurements; symbol sizes encompass ± SD unless shown otherwise by the error bars. Approximation of the initial velocities, normalized to units of enzyme, indicates 124-fold faster sulfation of 2-naphthol compared with 3-hydroxypyridine.

preincubations with PST for 30 min at a concentration of 1 mM, pyridoxal, pyridoxamine, and pyridoxamine-5-phosphate decreased enzyme activities by only 17, 17, and 36%, respectively. Because PLP was the most potent inhibitor in this family of

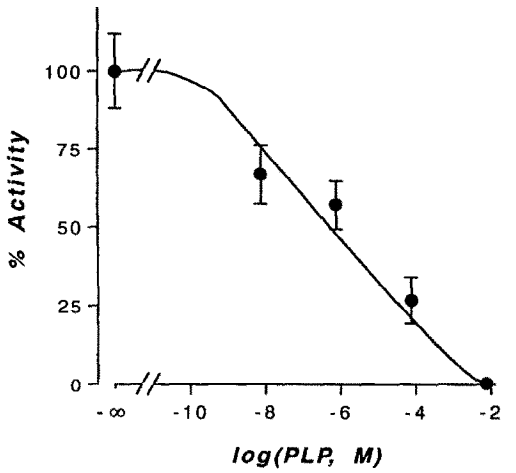

Fig. 4. Effect of varied PLP concentrations on apparent phenol sulfotransferase activity. Aliquots containing 0.16 μg enzyme were preincubated at 23° for 30 min with the indicated concentrations of PLP. These samples were then diluted 10-fold into assay reactions (10 min, 100 μM 2-naphthol, 188 μM PAPS). Values are means ± SD (N = 3), with the control assay rate (100%) being 6.23 pmol/min.

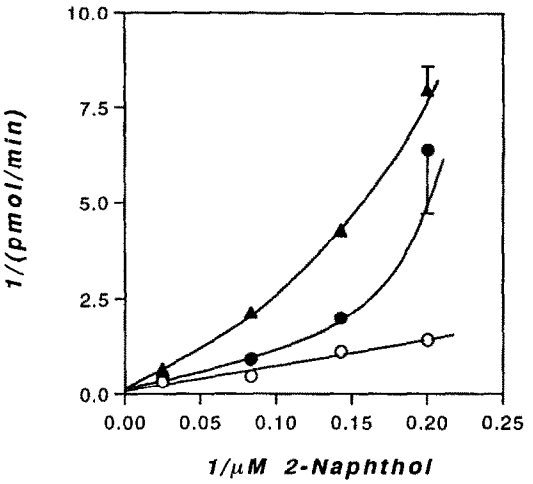

Fig. 6. Non-linear pattern of phenol sulfotransferase inhibition by pyridoxal-5-phosphate. Reactions were conducted for 15 min at 23°, pH 5.5, with 185 μM total PAPS. Varied initial concentrations of 2-naphthol are indicated. Concentrations of PLP were 0 (○), 1 μM (●) or 10 μM (▲). Each point is the mean of triplicate measurements; symbol sizes encompass ± SD unless shown otherwise by the error bars.

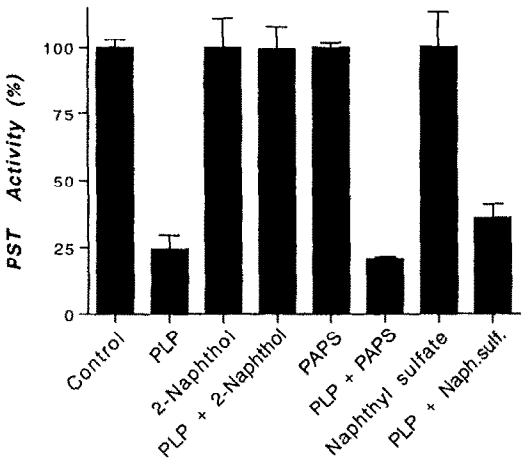

Fig. 5. Effects of phenol sulfotransferase substrates and products on inactivation by PLP. Aliquots containing 0.32 μg enzyme were preincubated at 23° for 30 min with the indicated compounds (PLP, 100 μM; 2-naphthol, 2-naphthyl sulfate, or PAPS, 500 μM). Samples were diluted 10-fold for assay of activity (8 min, 100 μM 2-naphthol, 184 μM PAPS). Values are means ± SD (N = 3), with the control assay rate (100%) being 5.97 pmol/min.

compounds, the remainder of the investigation was focused on PLP in particular.

Phenol sulfotransferases have two substrate binding sites; therefore, the abilities of the substrates or products to protect the enzyme from inactivation by PLP (100 μM) were tested (Fig. 5). Excess 2-naphthol (500 μM) completely prevented PLP inactivation, whereas PAPS (500 μM) was ineffective. There was a small amount of protection provided by naphthyl sulfate (500 μM), but this was not observed in the replicate experiment (data not shown). This indicates that PLP likely binds to the acceptor, or phenol, binding site on the enzyme.

To quantitate the inhibition of phenol sulfotransferase by PLP, standard steady-state kinetic analyses were conducted to determine K_i values and the inhibition pattern. In the absence of PLP, simple linear Lineweaver–Burk plots (1/v vs 1/2-naphthol) were obtained (Fig. 6 and Ref. 24). However, non-linear double-reciprocal plots were observed in the presence of PLP, and this prevented measurement of the K_i value (Fig. 6). Overall convergence toward the y-intercept is suggestive of competitive inhibition with respect to 2-naphthol, and this is consistent with the conclusion (Fig. 5) that PLP binds to the phenol site on PST.

One possible explanation for the above non-linear reciprocal plots was progressive inactivation of the phenol sulfotransferase during the assay. This would be most problematic at low 2-naphthol concentrations. To test this hypothesis, kinetic single progress curves were determined at varied 2-naphthol and PLP concentrations (Fig. 7). When product formation was measured under high 2-naphthol concentration (100 μM), the initial rate of reaction was inhibited 25% with 540 μM PLP. Under low 2-naphthol concentration (10 μM), however, 540 μM PLP completely inhibited further catalysis after 2 min; even 0.54 μM PLP decreased the rate of catalysis. These results are consistent with competition between PLP and 2-naphthol for binding to phenol sulfotransferase.

The progressive inhibition observed in the above single progress curves indicated that the inhibition

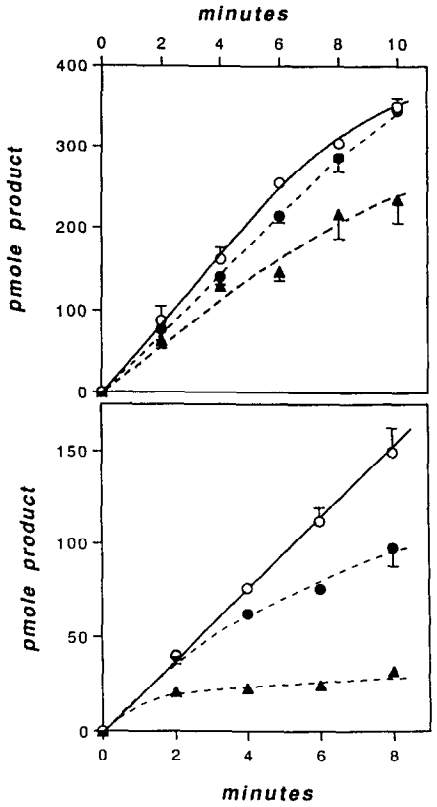

Fig. 7. Dependence of progressive inhibition of phenol sulfotransferase activity during catalysis on PLP and 2-naphthol concentrations. The reactions contained 0 (○), 0.54 μM (●), or 540 μM (▲) PLP. The upper panel contained 100 μM 2-naphthol, whereas the reactions for the lower panel contained 10 μM 2-naphthol. Each point is the mean of triplicate measurements; symbol sizes encompass ± SD unless shown otherwise by the error bars.

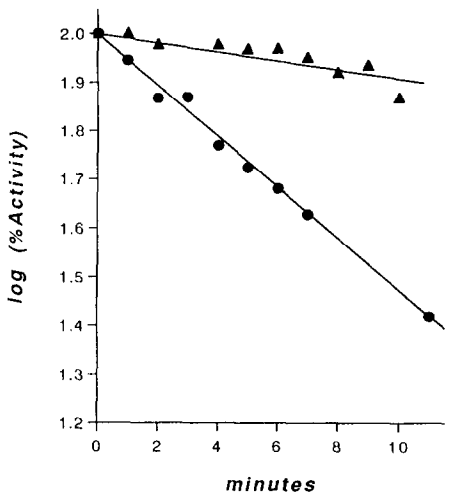

Fig. 8. Pseudo-first-order inactivation of phenol sulfotransferase by pyridoxal-5-phosphate. Purified enzyme plus PLP were incubated in Buffer A (Materials and Methods), with aliquots withdrawn at the indicated times for subsequent assay (100 μM 2-naphthol, 125 μM PAPS, pH 5.5, t = 1.5 min). Concentrations of PLP in the preincubations were 1 mM (●) and 10 μM (▲). Intermediate PLP concentrations are omitted for clarity. The uninhibited rate (100%) was 40 pmol/min.

of PST by PLP was time dependent with the formation of an inactive enzyme/PLP adduct. Therefore, rates of enzyme inactivation were measured (Fig. 8). Pseudo-first-order kinetics were observed over a wide range (10–1000 μM) of PLP concentrations, thereby allowing determination of apparent rate constants. Replot of these values in double-reciprocal fashion (Fig. 9) indicated a non-zero intercept, and this is consistent with the following kinetic mechanism:

$$\text{PST} + \text{PLP} \overset{K_I}{\rightleftharpoons} [\text{PST:PLP}] \overset{k}{\rightarrow} \text{PST-PLP}$$

where [PST:PLP] represents the initial dissociable complex, PST-PLP is the covalent adduct, K_I is the dissociation constant, and k is the first-order rate constant for the covalent reaction. These data indicate initial binding of PLP to PST (K_I = 23 μM), followed by a rate-limiting inactivation reaction (k = 0.077 min^{-1}). A replicate experiment gave similar results and values (K_I = 10 μM, k = 0.062 min^{-1}).

Two approaches were taken to examine the chemical nature of the reaction between phenol sulfotransferase and PLP. First, advantage was taken of the previously reported spectral changes exhibited by PLP after forming either Schiff base or thiohemiacetal adducts [32]. Upon incubation with equimolar enzyme, the absorbance of PLP was shifted toward 400 nm, along with decreased 325 nm and increased 300 nm absorbance (Fig. 10). These changes are entirely consistent with the formation of a PLP Schiff base adduct with the sulfotransferase.

The second approach to verify the type of reaction of PLP with PST took advantage of polyacrylamide gel electrophoresis. It is commonly reported that proteins exhibit decreased electrophoretic mobility upon phosphorylation, and we postulated that introduction of phosphate via a pyridoxal bridge could effect a similar change. Therefore, purified enzyme was preincubated with PLP with and without further treatment with excess borohydride. Electrophoresis subsequent to denaturation in the presence of dodecyl sulfate and 2-mercaptoethanol, which would easily reverse non-reduced Schiff base adducts, revealed a PLP plus borohydride-dependent decrease in mobility of the phenol sulfotransferase subunits (Fig. 11). Since no change was observed after reaction with either PLP or borohydride alone, these results support the spectral evidence that PLP binds to phenol sulfotransferase with the formation of a Schiff base. It should be noted that although these results indicate reversibility of the PST:PLP complex, which may seem at variance to the apparently irreversible patterns of kinetic inactivation of enzyme activity (Figs. 7–9), the conditions are dramatically different. The kinetic data apply to native enzyme, in which the rate constant for Schiff base hydrolysis may be negligible in comparison to its formation.

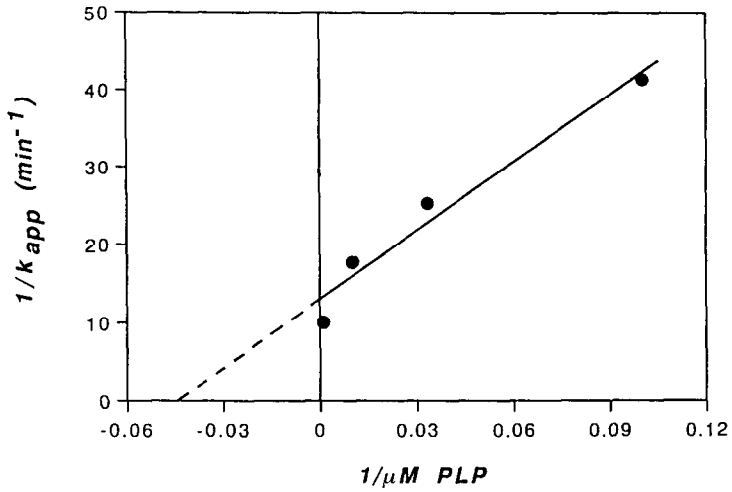

Fig. 9. Saturation kinetics for inactivation of phenol sulfotransferase by pyridoxal phosphate. Apparent first-order rates of inactivation (k_{app}) were determined as shown in Fig. 8, by multiplying the slopes of the semi-log plots by −2.303. The y-intercept indicates a limiting rate constant of enzyme inactivation of 0.077 min^{-1}, within a dissociable enzyme:PLP complex with $K_I = 23\ \mu M$.

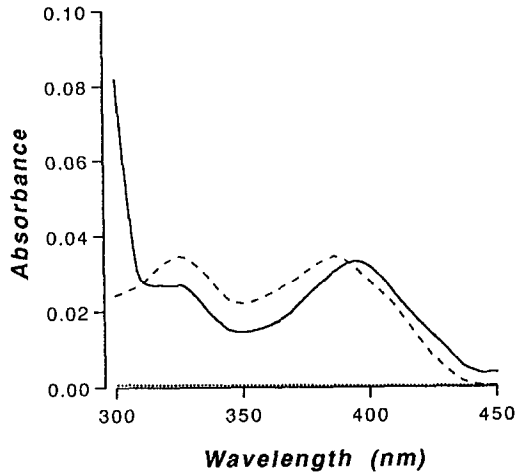

Fig. 10. Spectral evidence for the formation of a Schiff base adduct between phenol sulfotransferase and pyridoxal phosphate. After a 30-min incubation at 23° in Buffer A (Materials and Methods), absorbance spectra were measured for enzyme alone (0.24 mg/mL, or 7.5 μM subunits; dotted baseline), PLP alone (8 μM, dashed spectrum), or enzyme plus PLP (solid spectrum).

DISCUSSION

This investigation demonstrated that the biologically abundant compound, pyridoxal-5-phosphate (PLP), is a potent inhibitor of a phenol sulfotransferase. The evidence for the interaction is derived from enzyme kinetic, spectral, and electrophoretic approaches. The results all indicate that PLP binds to the enzyme within the phenol binding site followed by covalent linkage to the enzyme via a Schiff base adduct. Although we have not examined the pH dependence of this interaction, it is clear that inactivation can occur under both the acidic assay pH of 5.5 (Figs. 6 and 7) and at neutral pH of 7.4 (Figs. 8–11). This observation implicates the presence of a nucleophile, probably a lysyl residue, which remains unprotonated within the active site of the enzyme even under overall acidic conditions. Whether this group functions as a general base in the catalytic mechanism of the enzyme can only be speculated on, given the present data.

The structure of PLP (Fig. 1, V) has several features that probably contribute to its inhibitory potency. Most obvious is the 3-hydroxy moiety that may mimic a phenolic hydroxyl group. The 2-methyl group likely enhances binding, as this group in this position has been reported to decrease the K_m of human thermostable P-PST for phenol 10-fold [12]. It is not clear how 4-carboxaldehyde contributes to initial binding, although this group appears to be responsible for enzyme inactivation by covalent modification. Finally, the 5-(phosphonooxy)methyl group contributes a negative charge via a short methyl bridge. This spacing and rotational flexibility may be sufficient to enable this group to interact with a positively charged residue on the enzyme. Such a site is very likely, given the negative charges associated with the PAPS cosubstrate. It will be of interest to determine the nature of these interactions by molecular modeling once structural data are available.

Previous investigations have indicated several reactive nucleophiles within the active sites of phenol sulfotransferases [11, 15, 16, 33, 34]. To date, one of the most potent irreversible inhibitors is ADP-dialdehyde, which binds to the PAPS site on the enzyme [34]. This and other adenosine nucleotide-based dialdehydes exhibit maximal first-order rates of inactivation of 0.048–0.101 min^{-1}, with K_I values of 177–1540 μM [16]. By comparison, these rates are

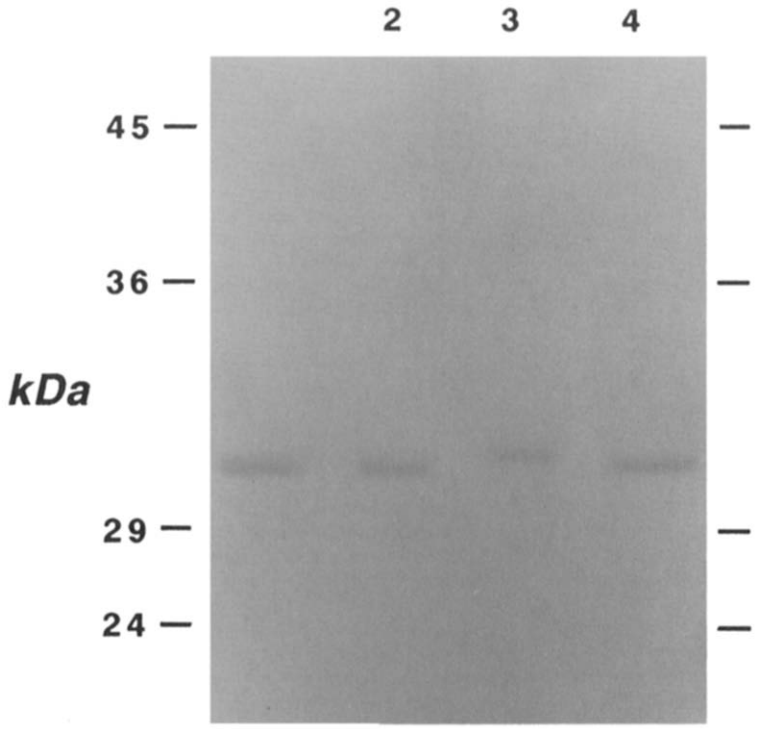

Fig. 11. Electrophoretic evidence for the formation of a Schiff base adduct between phenol sulfotransferase and pyridoxal phosphate. Aliquots of enzyme (1 μg) were incubated for 30 min in the absence (lanes 1 and 4) or presence (lanes 2 and 3) of 330 μM PLP, followed by 30 min in the absence (lanes 1 and 3) or presence (lanes 3 and 4) of 1.2 mM sodium borohydride.

very similar to those measured with PLP in this investigation, although the initial reversible binding of PLP to the enzyme was 10-fold greater. 1- and 2-Naphthaldehydes have been reported recently as inhibitors of rat liver phenol sulfotransferase IV, with K_i values of 29 and 89 μM, respectively [35]. Simple competitive inhibition with respect to 2-naphthol was observed, however, with no time dependence as observed in this investigation with PLP.

The inhibition of phenol sulfotransferase by pyridoxal phosphate may extend to other members of this family of enzymes. Mouse brain galactocerebroside sulfotransferase activity is sensitive to submillimolar concentrations of PLP [36]. An apparently distinct glucuronylglycolipid sulfotransferase is also inhibited by PLP, but is less sensitive than the related galactocerebroside sulfotransferase [37]. In neither case were the inhibitions closely examined by the methods described in this report, so it is impossible to compare the mechanisms of the PLP inhibition of these enzymes in greater detail.

Considerable effort has been made in the attempt to discover potent inhibitors of phenol sulfotransferases (see above) and sulfotransferases in general. Unfortunately, the most potent of these compounds, such as the polychlorophenols [38], exhibit considerable toxicity. The observation that PLP, and to a lesser extent its precursors, is inhibitory suggests a wide number of future investigations into the nutritional controls of metabolic processes known to be mediated by sulfotransferases. Examples include carcinogenesis [39–41], steroid metabolism and related endocrine effects [42], and catecholamine and neurotransmitter inactivation [10], to name a few. The observations in this report should be extended to the other members of this enzyme family, in order to determine the specificity or uniformity of this potentially important approach.

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