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Sulfation of Benzylic Alcohols Catalyzed by Aryl Sulfotransferase IV

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SUMMARY

Aryl sulfotransferase (AST) IV catalyzes the 3′-phosphoadenosine 5′-phosphosulfate-dependent sulfation of a variety of benzylic alcohols. Several molecular characteristics of benzylic alcohols were investigated for their ability to influence the catalytic efficiency of a homogeneous preparation of rat hepatic AST IV. The results of these studies indicated that lipophilicity of the benzylic alcohol was a major factor in determining catalytic efficiency, as represented by the values of k_{cat}/K_m. Furthermore, this effect was primarily mediated by a decrease in the apparent K_m as a function of increasing lipophilicity of the molecules. This effect of lipophilicity was documented by a linear correlation between the apparent K_m values of the benzylic alcohols and the logarithms of their octanol/water partition coefficients. In contrast to previously observed effects of *para* substituents on phenols, electronic effects of substituents on the phenyl ring had no effect on the catalytic efficiency of the enzyme with benzylic alcohols. In a further difference between phenol and benzylic alcohol substrates for AST IV, 2-naphthol exhibited pronounced substrate inhibition at pH 7.0, whereas the analogous benzylic alcohol, 2-naphthalenemethanol, did not. Stereochemistry at the benzylic carbon also had an effect on the catalytic efficiency of the AST IV; k_{cat}/K_m values for *S*-(−)-1-phenylethanol were approximately 3-fold higher than for *R*-(+)-1-phenylethanol.

Sulfation is a metabolic reaction which governs various toxicities exhibited by drugs, carcinogens, and other xenobiotics containing phenolic, benzylic alcohol, arylhydroxamic acid, and arylhydroxylamine functional groups. In general, sulfate esters of phenols are more polar, more readily excreted, and less toxic than the parent phenols. However, sulfate esters of benzylic alcohols, arylhydroxamic acids, and arylhydroxylamines are often more toxic than phenyl sulfates due to enhanced chemical reactivity with cellular constituents. The chemical reactivity of these sulfate esters results from the ability of HSO_4^- to act as a leaving group, with resonance stabilization of the resulting benzylic carbocation or arylnitrenium ion. The involvement of sulfation in the conversion of benzylic alcohols, arylhydroxamic acids, and arylhydroxylamines into electrophilically reactive metabolites has been recently reviewed (1). Representative examples of benzylic alcohols that are metabolized to electrophilic sulfate esters include 1′-hydroxysafrole (2, 3), 7-hydroxymethyl-12-methylbenz[*a*]anthracene (4, 5), 1′-hydroxy-2′,3′-dehydroestragole (6), 2-methylbenzyl alcohol (7), and 1-naphthalenemethanol (8).

Previous studies (9) have shown that homogeneous AST IV from rat liver catalyzes the sulfation of a benzylic alcohol, 1-naphthalenemethanol. The pH optimum for this reaction is 6.5–7.0, and the AST IV-catalyzed sulfation of 1-naphthalenemethanol is inhibited by the AST inhibitor, pentachlorophenol (9). Prior to these studies on benzylic alcohol sulfation, the specificity of AST IV for sulfation of phenols, organic hydroxamic acids, catecholamines, and tyrosine esters was described (10, 11). Furthermore, the mechanism of AST IV-catalyzed sulfation of phenols has been investigated (12, 13). Although there have been extensive studies on sulfation of phenols catalyzed by the purified AST IV, structural features affecting substrate specificity of AST IV for benzylic alcohols have not been previously investigated.

The reaction catalyzed by AST IV in the sulfation of benzylic alcohols is seen in Eq. 1,

$$\text{Ar-CH}_2\text{-OH} + \text{PAPS} \leftrightarrows \text{Ar-CH}_2\text{-OSO}_3^- + \text{PAP} \qquad (1)$$

where Ar represents an aryl substituent and PAP represents adenosine 3′,5′-bisphosphate. The present study was conducted in order to determine which molecular characteristics of the aryl substituent are responsible for the specificity of AST IV with benzylic alcohol substrates. Furthermore, since the benzylic carbon can have substituents other than hydrogen, the potential for stereochemical control of substrate specificity was also investigated. Model substrates have been used to provide insight into the structural features responsible for activity of benzylic alcohols as substrates for AST IV.

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ABBREVIATIONS: AST, aryl sulfotransferase; PAPS, 3′,5′-phosphoadenosine 5′-phosphosulfate.

Materials and Methods

Substrates and products. All benzylic alcohols used as substrates for the studies on specificity of AST IV were obtained from Aldrich Chemical Co., and purity was confirmed by thin layer chromatography and high performance liquid chromatography. Optical purity of 1-phenylethanols was confirmed by polarimetry on a Perkin-Elmer model 141 polarimeter; specific rotations of the *R*-(+)- and *S*-(−)-enantiomers were +43.9° and −42.5°, respectively (determined in methylene chloride at 589 nm and 29.1°). PAPS was prepared by chemical synthesis (14), and [^{35}S]PAPS was obtained from New England Nuclear. All other assay reagents and buffer components were obtained from commercial sources.

Stability of the product benzylic sulfates under the conditions of enzyme assay was determined after chemical synthesis of benzyl sulfate and 1-naphthalenemethyl sulfate by published procedures (8). These benzylic sulfates were not hydrolyzed during incubation at 37° under the conditions of enzyme assay. This was determined by thin layer chromatography of the sulfate esters on silica gel with ethanol/hexanes (1:1) following incubation with assay components for 10 min. Furthermore, similar control experiments confirmed that the benzylic sulfates were not hydrolyzed in the chromatography solvent that was used for analysis of reaction mixtures for sulfate ester formation [i.e., 1-propanol/29% ammonium hydroxide/water (6:3:1) at 4° for 1.5 hr].

AST IV. Hepatic AST IV was purified to homogeneity from male Sprague-Dawley rats using a modification (9) of a published procedure (10). Analytical isoelectric focusing was utilized as a final method for confirmation of homogeneity, since this is the most effective means of determining contamination by other isoenzymes of AST (10, 15). Isoelectric focusing was carried out in 4.8% (w/v) polyacrylamide gels using a final ampholyte (Servalyt 3-10, Serva Biochemicals) concentration of 2.0% (w/v).

Benzylic alcohol sulfation. The assay used for sulfation of benzylic alcohols catalyzed by AST IV was a modification of the method described by Sekura and Jakoby (10). All assays were conducted at pH 7.0. This is within the optimum pH range for the AST IV-catalyzed reaction with benzyl alcohol (6.3–7.0);[1] the reaction with 2-naphthalenemethanol has pH optima at approximately 6.2 and 7.0.[1] Reaction mixtures of 0.03 ml contained 0.25 M potassium phosphate at pH 7.0, 8.3 mM 2-mercaptoethanol, 0.2 mM [^{35}S]PAPS (27–54 Ci/mol), 5% (v/v) acetone, and various concentrations of the alcohol studied. Reactions were initiated by the addition of AST IV, incubated at 37°, and terminated after 10 min by the addition of 5 μl of 3.0 M acetic acid. An aliquot (20 μl) of the reaction mixture was then applied to a cellulose thin layer chromatogram (Eastman Kodak) and developed with 1-propanol/29% ammonium hydroxide/water (6:3:1) at 4° until the solvent had moved approximately 8 cm. The product benzylic sulfates had R_f values greater than 0.9 under these conditions. The amount of product formed in the reaction was determined by liquid scintillation analysis of a 2-cm segment of the chromatogram that included the solvent front. V_{max} and apparent K_m values were determined by graphical analysis of 1/(initial velocity) versus 1/(concentration of benzylic alcohol) using a linear least squares fit of the data. At least five concentrations of each benzylic alcohol were used and these included concentrations both greater than and less than the apparent K_m.

Partition coefficients of benzylic alcohols. Partition coefficients were determined by allowing the various alcohols to partition between equal volumes of 0.25 M potassium phosphate in water at pH 7.0 (presaturated with 1-octanol) and 1-octanol (presaturated with the phosphate buffer). Alcohols were dissolved in the aqueous phase at 0.5–1.0 mg/ml and were allowed to partition between the phases by shaking in sealed glass containers at 25° for 72 h. The concentration of the benzylic alcohol in each phase was determined by ultraviolet absorbance.

[1] S. I. Rao and M. W. Duffel, unpublished observations.

Results

Substrate specificity with benzylic alcohols. Various benzylic alcohols were tested as substrates for the homogeneous AST IV. The kinetic constants for several of these alcohols are summarized in Table 1, with the corresponding data for phenol listed for comparison. The apparent K_m values exhibited considerable variation, ranging from 0.012 mM for 1-naphthalenemethanol to 0.96 mM for 2-furanmethanol. In contrast to previous studies with substituted phenols (10–12), there was little change in the maximal velocity of sulfation with changes in the chemical structure of the benzylic alcohol.

Since the variation in apparent K_m for the benzylic alcohol substrates appeared to follow the hydrophobic character of the substrate, this hypothesis was tested using octanol/water partition coefficients for the benzylic alcohols. As seen in Fig. 1, the apparent K_m values decreased slightly (r = 0.90) with increasing logarithms of the corresponding partition coefficients. No correlation of σ constants for electron-donating and withdrawing substituents was possible when these constants were plotted versus log K_m, log V_{max}, or log K_{cat}/K_m.

V_{max} values were also determined using a previously described method (12, 16) for varying PAPS and the benzylic alcohol in a constant ratio. The V_{max} values (nmol of product formed/min/mg of AST IV) obtained using this method were as follows: benzyl alcohol (27.3), 4-chlorobenzyl alcohol (67.3), 4-methylbenzyl alcohol (63.2), 4-nitrobenzyl alcohol (67.0), and 4-trifluoromethylbenzyl alcohol (29.8). Regardless of the method utilized for calculation of V_{max}, there was no correlation between

TABLE 1
AST IV specificity for benzylic alcohols[a]

Substrate	K_m (app)[b]	V_{max}	k_{cat}/K_m
	mM	nmol/min/mg AST IV	min^{-1} mM^{-1}
Benzyl alcohol	0.790	22.5	1.7
4-Methylbenzyl alcohol	0.189	25.1	8.1
4-Nitrobenzyl alcohol	0.199	20.6	6.3
1-Naphthalenemethanol	0.012	19.0	97
2-Naphthalenemethanol	0.026	77.6	182
2-Furanmethanol	0.960	10.2	0.6
Phenol	0.030	89.9	183

[a] Kinetic constants were determined as described under Materials and Methods, using homogeneous AST IV.
[b] The K_m was determined with a single concentration of PAPS (0.2 mM) and is thus labeled an apparent K_m.

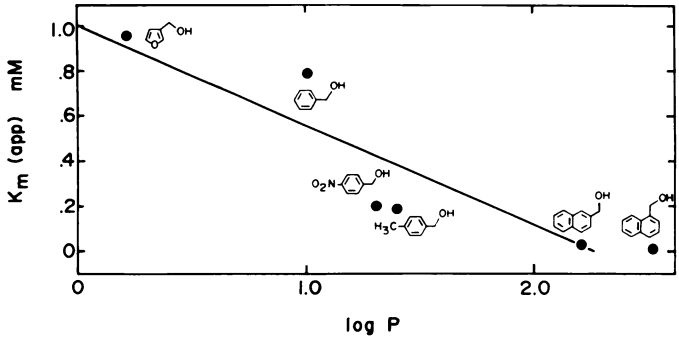

Fig. 1. Effect of the lipophilicity of benzylic alcohols on the apparent K_m values for sulfation catalyzed by AST IV. Octanol/water partition coefficients (P) and apparent K_m values were determined as described under Materials and Methods.

V_{max} and any quantitative parameters for lipophilicity or electronic effects of substituents.

Stereochemistry of benzylic alcohol sulfation. The effect of stereochemistry on benzylic alcohol sulfation catalyzed by AST IV was also examined. The model substrates for this study were *R*-(+)-1-phenylethanol and *S*-(−)-1-phenylethanol. Kinetic constants for these enantiomers are seen in Table 2. While the effect of the stereochemical configuration of the benzylic hydroxyl group on the kinetic constants for sulfation catalyzed by AST IV was small, there was an overall difference in the catalytic efficiency of the enzyme with the two enantiomers. The enzyme catalyzed sulfation of the *S*-(−)-enantiomer with a 3-fold greater efficiency as evidenced by k_{cat}/K_m values.

Comparison of initial rate kinetics for 2-naphthol and 2-naphthalenemethanol. Since substrate inhibition has been observed for purified ASTs with several phenolic substrates (10–12, 15), initial rate kinetics for 2-naphthol and the corresponding benzylic alcohol, 2-nephthalenemethanol, were compared at pH 7.0. The results in Fig. 2 indicate that substrate inhibition with 2-naphthol was evident at substrate concentrations above 6 μM, whereas no substrate inhibition was detected with 2-naphthalenemethanol. The apparent K_m for 2-naphthalenemethanol under these conditions was 26 μM.

Discussion

Sulfation is an important biotransformation for detoxication of some benzylic alcohols and for generation of chemically reactive, and more toxic, metabolites from other benzylic alcohols. AST IV from rat liver catalyzes the sulfation of a variety of benzylic alcohols. Our results with model substrates indicate that lipophilicity is a major determinant of the ability of benzylic alcohols to serve as substrates for AST IV. Furthermore, the lipophilicity of the benzylic alcohol has its major effect on binding of the alcohol to the enzyme, since V_{max} values are not appreciably affected by changes in lipophilicity when compared to the much larger changes in apparent K_m values. While electron-donating and -withdrawing substituents have been shown to affect the V_{max} for phenol sulfation (12), electronic effects of substituents on the aromatic ring did not influence the AST IV-catalyzed sulfation of benzylic alcohols.

Stereochemical considerations may also be important in determining the specificity of AST IV for benzylic alcohol substrates. In the case of 1-phenylethanol, however, the stereochemical preference was not absolute. Nevertheless, the K_{cat}/K_m values indicated that catalytic efficiency with the *S*-(−)-enantiomer was higher than with the *R*-(+)-isomer.

In addition to substituent effects and stereochemistry, substrate inhibition was examined as a potential modulator of AST IV-catalyzed sulfation of benzylic alcohols. Previous studies had described substrate inhibition of AST IV at pH 5.5 by phenol substrates (10). Our results showed a marked difference between 2-naphthol and the analogous benzylic alcohol, 2-naphthalenemethanol. Indeed, there was no substrate inhibition observed with 2-naphthalenemethanol as substrate for AST IV. Additionally, the severe nature of the substrate inhibition observed with 2-naphthol at pH 7.0 may help to explain the basis for using differential assay pH to estimate AST isoenzyme content in partially purified preparations. That is, at the usual assay concentrations of 2-naphthol for determination of ASTs I and II at pH 7.5 (15), AST IV would be subject to severe substrate inhibition. However, AST IV could still be very important in metabolism of low concentrations of 2-naphthol at pH 7.0–7.5 due to its much lower apparent K_m than that for ASTs I and II. This interpretation is consistent with studies on rat hepatic cytosol which indicate differential substrate inhibition effects for 4-nitrophenol sulfation as a function of pH (17).

TABLE 2

Effect of stereochemistry on sulfation of 1-Phenylethanols*

Enantiomer	K_m (app)	V_{max}	k_{cat}/K_m
	mM	nmol/min/mg AST IV	$min^{-1}\ mM^{-1}$
R-(+)-1-Phenylethanol	2.0	14.4	0.4
S-(−)-1-Phenylethanol	1.2	23.1	1.2

* Kinetic constants were determined as described under Materials and Methods, using homogeneous AST IV.

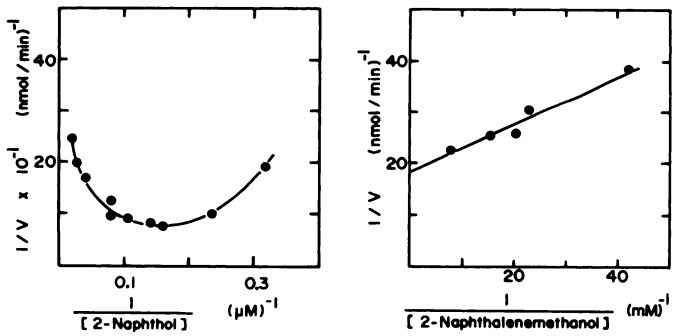

Fig. 2. Initial rate kinetics for sulfation of 2-naphthol and 2-naphthalenemethanol catalyzed by AST IV. Assays were conducted as described under Materials and Methods.

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