Specificity of Alcohol Dehydrogenases for Sulfoxides†

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Received October 31, 1997; Revised Manuscript Received February 4, 1998

ABSTRACT: Sulfoxides inhibit horse liver alcohol dehydrogenase (*Eq*ADH) by binding to the enzyme−NADH complex. X-ray crystallography suggests that sulfoxides make a cation−π interaction with the benzene ring of Phe-93 [Cho et al. (1997) *Biochemistry 36*, 382−389]. Structure−function relationships were examined with seven different sulfoxides binding to five human enzymes (α, β_1, γ_2, π, and σ) and three mutated forms of the horse enzyme. The human γ_2 enzyme, *Eq*ADH, and *Eq*ADH with Phe-93 replaced with Trp were selectively and strongly inhibited ($K_i \leq 1$ μM) by the 3-butyl or hexyl derivatives of thiolane 1-oxide. The other human enzymes (all with Thr-48) and *Eq*ADH with Ser-48 substituted with Thr had relatively lower affinities for the thiolane 1-oxides due to close contact of the methyl group of Thr-48 with a carbon adjacent to the sulfoxide sulfur. *Eq*ADH binds the *S* isomers of 3-butylthiolane 1-oxides, hexyl methyl sulfoxide, and phenyl methyl sulfoxide more tightly than the *R* isomers, but *Eq*ADH with Phe-93 substituted with Ala and the human α enzyme (with Ala-93) prefer (*R*)-phenyl methyl sulfoxide, apparently because the phenyl ring fits into the space near residue 93. *Eq*ADH and the enzymes with Phe-93 replaced with Ala or Trp had similar affinities for sulfoxides, indicating that the contribution of the cation−π interaction to binding is small or compensated for by altered interactions. *Ab initio* calculations also suggest that the interaction of a sulfoxide with benzene is relatively weak.

Sulfoxides are analogues of ketones, the products of the oxidation of secondary alcohols by ADH,[1] and they bind preferentially to the enzyme−NADH complex. They are uncompetitive inhibitors against varied concentrations of alcohols and can inhibit ADH in the presence of saturating concentrations of alcohols (*1*, *2*). Such inhibitors could be more effective than competitive inhibitors, such as ethanol and 4-methylpyrazole, for prevention of toxicity caused by methanol and ethylene glycol metabolism. Studies on the specificities of alcohol dehydrogenases for sulfoxides should define the binding interactions that make sulfoxides potent inhibitors and could lead to useful therapeutic agents.

Five ADH enzymes (Table 1) in human tissues can function in metabolism of ethanol and other alcohols (*3*). The class I human ADHs (*Hs*ADH α, β, γ) have about 95% amino acid sequence identity to each other, 80−90% sequence identity to *Eq*ADH, and 60% and 69% sequence identity to *Hs*ADH π and σ, respectively (*4*). The three-dimensional structures of the human α, β, and σ enzymes and *Eq*ADH are very similar (*4*−*6*), but the amino acid residues in the active sites and the specificity and stereoselectivity for substrates and inhibitors differ.

The participation of active site residues in the binding of sulfoxides was studied with human enzymes and with mutants of *Eq*ADH. The hydroxyl group of Ser-48 forms a hydrogen bond to the sulfoxide oxygen, which coordinates to the catalytic zinc. Several hydrophobic residues in the substrate binding pocket make van der Waals contacts with the side chains of the sulfoxides. The electron-rich π cloud of the benzene ring of Phe-93 interacts with the positively charged sulfur in enzyme−NADH−sulfoxide complexes (*7*). The noncovalent ion−quadrupole interaction is important in biological recognition, for instance in the interaction of acetylcholine with acetylcholine esterase and of *S*-adenosylmethionine with DNA methyltransferase (*8*−*10*). The contribution of the cation−π interaction to the binding of sulfoxide inhibitors by ADH was tested by substituting Phe-

Table 1: Amino Acid Residues in the Substrate Binding Sites of ADHs[a]

		human				
		class I			class II	class IV
residue no.	horse E	*ADH1* α	*ADH2* β	*ADH3* γ	*ADH4* π	*ADH7* σ
48	S	T	T	S	T	T
57	L	M	L	L	F	M
93	F	A	F	F	Y	F
110	F	Y	Y	Y	L	L
116	L	V	L	L	L	I
117	S	S	G	G	S	Δ[b]
140	F	F	F	F	F	F
141	L	L	L	V	F	M
143	T	I	T	V	T	T
294	V	V	V	V	V	V
306	M	M	M	M	E	M
309	L	L	L	L	I	F
318	I	I	V	I	F	V

[a] From refs 45 and 46. [b] A deletion of one residue.

† This work was supported by United States Public Health Service Grant AA00279 (to B.V.P.) and a predoctoral fellowship from The University of Iowa Center for Biocatalysis and Bioprocessing (to H.C.).

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[1] Abbreviations: ADH, alcohol dehydrogenase; CD, circular dichroism; *Eq*ADH or E, EE isoenzyme of horse (*Equus caballus*) liver ADH; F93A, mutation of Phe-93 to Ala; F93W, mutation of Phe-93 to Trp; S48T, mutation of Ser-48 to Thr; *Hs*ADH, human (*Homo sapiens*) ADH; BTO, 3-butylthiolane 1-oxide; HF, Hartree−Fock.

S0006-2960(97)02704-9 CCC: $15.00
Published on Web 03/13/1998

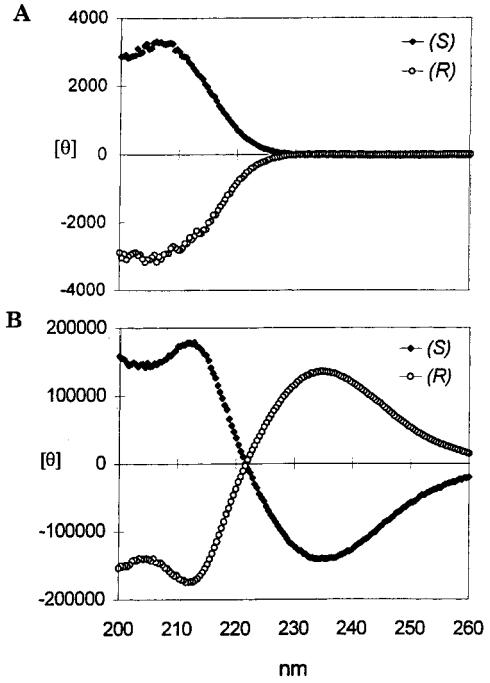

FIGURE 1: Cotton effects in CD spectra of stereoisomers of (A) hexyl methyl sulfoxide and (B) phenyl methyl sulfoxide in water. The CD scans were made with 0.2-nm intervals with an AVIV CD spectrometer 62Ds at 25 °C. Background absorbance from the solvent was digitally subtracted. Molar ellipticity ([θ]) was calculated using the equation $[\theta] = 10\theta/dC$, where d is the path length in dm and C is the molar concentration.

93 with alanine or tryptophan residues and by calculating complexation energies of dimethyl sulfoxide and aromatic compounds.

EXPERIMENTAL PROCEDURES

Materials. Crystalline horse liver ADH (EE isoenzyme), NAD^+, and NADH were purchased from Boehringer Mannheim. Sulfoxides were either purchased (Aldrich) or synthesized (*2*). Clones for expression of *Hs*ADH α (*11*), β_1 (*12*), and σ (*13*) enzymes were obtained from Dr. Thomas D. Hurley (Indiana University School of Medicine) and for γ_2 (*14*) and π (*15*) enzymes from Dr. Jan-Olov Höög (Karolinska Institutet).

Chromatography and Characterization of Stereoisomeric Sulfoxides. The stereoisomers of the sulfoxides were separated isocratically on a Chiralcel OB-H column (cellulose tribenzoate, Chiral Technologies Inc., 4.6 mm × 250 mm). Methyl phenyl sulfoxides were separated (selectivity α = 1.87) with hexane/2-propanol (90/10, v/v) at a flow rate of 1 mL/min with detection at A_{254} and methyl hexyl sulfoxides with hexane/2-propanol (95/5, v/v) at 0.5 mL/min, detected at A_{214} (α = 1.10). The concentrated fractions were rechromatographed to confirm enantiomeric purity. The concentrations of sulfoxides were determined using UV spectrophotometry (ϵ_{210} in water = 820 M^{-1} cm^{-1} for BTO and hexyl methyl sulfoxide, ϵ_{230} in water = 3370 M^{-1} cm^{-1} for phenyl methyl sulfoxide).

The *S* isomer of phenyl methyl sulfoxide elutes faster than the *R* isomer on Chiralcel OB-H (*16*). The first peak of hexyl methyl sulfoxide showed a positive Cotton effect in its CD spectrum (Figure 1), just like the *S* enantiomer of butyl methyl sulfoxide (*17*), and therefore is assigned as the *S* enantiomer. The λ_{max} based on CD is at 208 nm for hexyl methyl sulfoxide and at 212 and 235 nm for phenyl methyl sulfoxide. The *R* isomer of phenyl methyl sulfoxide showed a positive exciton-coupled (n → π^* and π → π^*) Cotton effect around 235 nm, which is correlated to the absolute configuration of alkyl aryl sulfoxides.

Expression and Purification of Alcohol Dehydrogenases. The mutated *Eq*ADHs were expressed and purified using the procedure described by Park and Plapp (*18*). The human enzymes were prepared essentially as described by Stone et al. (*19*) and Hurley et al. (*6*). Protein homogeneity was established by polyacryamide gel electrophoresis in the presence of sodium dodecyl sulfate. The specific absorbances for *Hs*ADH π (A_{280} = 0.570 mg^{-1} cm^2) and σ (A_{280} = 0.490 mg^{-1} cm^2) were calculated from the extinction coefficients of the constituent amino acids and corrected for hyperchromic effects by reference to the values for *Eq*ADH (calculated A_{280} = 0.40 mg^{-1} cm^2, 0.455 mg^{-1} cm^2 observed). For class I *Hs*ADHs (α, β, γ), A_{280} = 0.455 mg^{-1} cm^2 was used. The turnover numbers based on a standard assay (1.75 mM NAD^+, 550 mM ethanol, 6.5 mM semicarbazide, 18 mM glycine, 85 mM sodium pyrophosphate, pH 9, 25 °C) were 0.56 s^{-1} (*Hs*ADH α), 0.093 s^{-1} (β_1), 0.61 s^{-1} (γ_2), 0.34 s^{-1} (π), and 27 s^{-1} (σ).

Kinetic Studies. Inhibition of the forward reaction was studied with varied concentrations of inhibitors against varied concentrations of ethanol with saturating NAD^+ (2 mM for *Hs*ADH σ and 1 mM for other enzymes) in 46 mM sodium phosphate buffer, pH 7, at 25 °C. For inhibition studies of the reverse reaction, cyclohexanone or freshly distilled acetaldehyde and saturating NADH (0.3 mM for *Hs*ADH σ and 0.1 mM for others) were used. Substrate concentrations were adjusted according to the K_m values of the enzymes (in the ranges of 0.2−2 mM ethanol for *Eq*ADH and E/F93W, 0.07−0.5 mM acetaldehyde for E/S48T, 2−15 mM ethanol or 0.09−0.9 mM cyclohexanone for E/F93A and *Hs*ADH α, 0.025−0.25 mM acetaldehyde for *Hs*ADH β_1, 0.2−2 mM ethanol for *Hs*ADH γ_2, 5−50 mM acetaldehyde for *Hs*ADH π, 15−150 mM ethanol or 6−60 mM acetaldehyde for *Hs*ADH σ enzymes). Initial velocities were determined from the change of absorbance due to NADH (ϵ_{340} = 6.22 mM^{-1} cm^{-1}). Data were fitted to the equation for uncompetitive inhibition ($v = VA/[K_m + A(1 + I/K_{ii})]$) for the forward reaction or competitive inhibition ($v = VA/[K_m(1 + I/K_{is}) + A]$) for the reverse reaction (*20*). Standard errors of the fitted values were usually in the range of 5−15%. The K_i values reflect binding to the enzyme−NADH complex. Some data sets were described better by noncompetitive inhibition, which can arise if the inhibitor also binds to the enzyme−NAD^+ complex, but the corresponding K_i values agreed within a factor of about 3 for the different fits.

Modeling and Computation. Binding of inhibitors with the ADHs was modeled using the program O (*21*). The close contacts between active site residues and inhibitors were measured after manual docking.

Ab initio molecular orbital calculations were performed on dimethyl sulfoxide, benzene, indole, and 1:1 complexes using the Gaussian 94 program on a Silicon Graphics Power Challenge computer (*22*). Geometry-optimized sulfoxides and benzene were superimposed onto the sulfoxide group of BTO and Phe-93 in the 1.66-Å resolution structure of

Table 2: Inhibition of Human Alcohol Dehydrogenases by Sulfoxides[a]

inhibitor	K_i, μM					
	*Eq*ADH	*Hs*-α	*Hs*-β_1	*Hs*-γ_2	*Hs*-π	*Hs*-σ
dimethyl sulfoxide	1500	120000	43000	1500	140000	130000
ethyl methyl sulfoxide	410	8600	13000	360	36000	24000
phenyl methyl sulfoxide	58[b]	65[b]	1400[b]	360	1000	11000
hexyl methyl sulfoxide	31[b]	590	370	11	10	22
thiolane 1-oxide	19[c]	7000	6800	15	2100	26000
3-butylthiolane 1-oxide	0.56	73	120	0.30	110	530
3-hexylthiolane 1-oxide	0.19[c]	11	14	0.35	15	42

[a] Inhibition of the forward or reverse reactions was studied with varied concentrations of inhibitors against varied concentrations of substrates in 46 mM sodium phosphate buffer, pH 7, at 25 °C. [b] Calculated for the racemic mixture from values in Table 4 using the relationship $2/K_{i(racemate)} = 1/K_{i(S)} + 1/K_{i(R)}$. [c] From refs 1 and 2.

*Eq*ADH−NADH−*SS*-BTO (PDB entry 3BTO), and the coordinates of the dimethyl sulfoxide−benzene complex were extracted. The coordinates of the complex of dimethyl sulfoxide with the indole ring of Trp-93 were obtained by docking of dimethyl sulfoxide into the active site of the structure of the *Eq*ADH/F93W−NAD−trifluoroethanol complex determined by X-ray crystallography at 2.5-Å resolution (S. Selveraj and S. Ramaswamy, unpublished). The complexation energies of NH_4^+, acetone, 2-methylpropane, and *cis*-*N*-methylformamide with benzene were also calculated for comparison with the dimethyl sulfoxide−benzene complex. Both geometry optimizations and energy calculations were carried out at the 3-21G, 6-31G**, and 6-31+G** levels. Geometry optimization with HF/6-31G** was followed by correlation energy calculation with B3LYP or B3PW91 of the density functional theory method (*23*−*25*). The program SYBYL (Tripos Associates, ref *26*) was used to view the minimized structures.

RESULTS AND DISCUSSION

Inhibition of Human Enzymes. The inhibitory activity of sulfoxide compounds was surveyed with five human enzymes (Table 2). The γ enzyme is selectively inhibited by sulfoxide inhibitors, and the binding affinities are similar to those for *Eq*ADH. Inhibition of *Hs*ADH α, β, π, and σ by most sulfoxides is 2 or 3 orders of magnitude lower than inhibition of *Eq*ADH. As the length of the alkyl chain of the sulfoxide increases, the binding affinities increase with all enzymes. Extension of the butyl chain of the 3-substituted thiolane 1-oxide to hexyl has small effects on inhibition of *Eq*ADH and *Hs*ADH γ. The added methylene units apparently do not make good van der Waals contacts due to the relatively wide substrate binding channel. Generally, *Hs*ADH π and σ are more sensitive to the length of the alkyl chain.

The binding constants of hexyl methyl sulfoxide to the π and σ enzymes are about the same as those for *Eq*ADH or *Hs*ADH γ, even though the binding constants of other sulfoxides with π and σ enzymes are relatively high compared to those with *Hs*ADH γ. The interaction between the hexyl group and the middle part of the substrate binding site of the π enzyme could be tighter than in other enzymes because the site in the π enzyme is expected (no three-dimensional structure of the π enzyme is available at present) to be narrower than in the other enzymes (*27*). The middle part of the binding site of the σ enzyme is also narrow, as it has Met-57, Met-141, and Phe-309 instead of leucines. The modeled structure shows good van der Waals contacts between the alkyl chains of hexyl methyl sulfoxide or BTO and Val-294, Met-306, Phe-309, and Ile-116 (Figure 2A). However, thiolane 1-oxide and 3-substituted thiolane 1-oxide are relatively poor inhibitors for the σ enzyme, probably due to the close contact between C4 of thiolane 1-oxide and the side chain of Met-141.

The K_i of phenyl methyl sulfoxide with the α enzyme is about the same as for *Eq*ADH, apparently because the increased space near residue 93 (Ala-93 instead of Phe) can accommodate the phenyl group.

The strong inhibition of *Eq*ADH and *Hs*ADH γ as compared to the other enzymes probably results from the presence of Ser-48 instead of Thr. Steric hindrance between the sulfoxide and the methyl group of Thr-48 apparently decreases the binding affinities of the sulfoxides. Enzymes with Thr-48, except *Hs*ADH α, also show low activity on secondary alcohols due to this steric hindrance (*28*−*30*). For these human enzymes, formamide derivatives would be better inhibitors since they are not hindered by Thr-48 (*31*).

Determinants of Specificity for Binding of Sulfoxides. The structure−function relationships were explored further with the horse enzyme where the effects of mutations can be studied against a common structural background. In the human enzymes, multiple differences in amino acid residues can produce subtle changes in structure that make interpretations uncertain. The effects of S48T, F93A, or F93W single mutations in *Eq*ADH on the binding of sulfoxide inhibitors were studied (Table 3). Ser-48 is located at the top of the substrate binding site, and the methyl group introduced by the S48T mutation is close to C1 of substrates. The E/S48T enzyme has about 10−100-fold lower affinity for sulfoxides than wild-type enzyme does, probably due to steric hindrance between the methyl group of Thr-48 and the carbon next to sulfur, as the distance is 2.8 Å in a modeled structure (Figure 2A).

The F93A mutation was used to evaluate both structure−function relationships and the possible cation−π interaction of the sulfoxides with Phe-93. The smaller sulfoxides, dimethyl sulfoxide, ethyl methyl sulfoxide, and thiolane 1-oxide (tetramethylene sulfoxide), bind to *Eq*ADH and E/F93A enzymes with the same selectivity. In contrast, BTO and 3-hexylthiolane 1-oxide are relatively weaker inhibitors of E/F93A enzyme. The small sulfoxides could bind to E/F93A enzyme in various orientations and fill the space vacated by removal of the Phe-93 side chain, producing hydrophobic interactions that could compensate for the lost interaction with Phe-93 and maintain the inhibitory potency. Likewise, the *R* isomer of phenyl methyl sulfoxide could bind in another mode, and its phenyl group could fill the

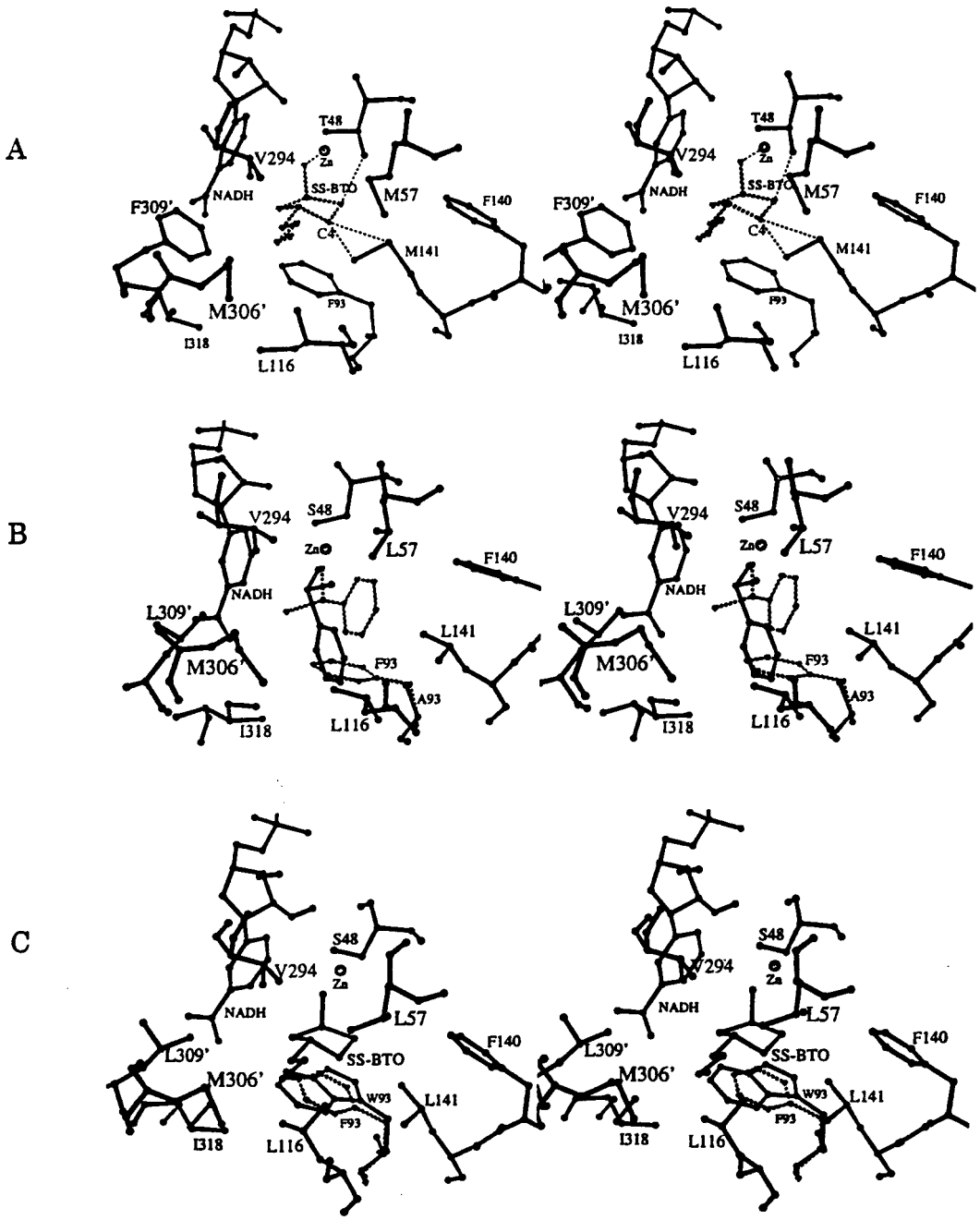

FIGURE 2: Models of interactions between sulfoxide inhibitors and active site residues. (A) Binding of (1*S*,3*S*)-3-butylthiolane 1-oxide (*SS*-BTO) to *Hs*ADH σ enzyme. Small adjustments in the position of Met-141 (*4*) were made in order to get the fewest close contacts. The close contacts with Thr-48 and Met-141 are indicated by dotted lines. (B) (*R*)-Phenyl methyl sulfoxide binding to *Eq*ADH (dotted phenyl methyl sulfoxide and Phe-93) or E/F93A. The coordinates of E/F93A were obtained from the coordinates of the *Eq*ADH−NAD^+−pentafluorobenzyl alcohol complex (*51*) through graphical mutation. (C) (1*S*,3*S*)-3-Butylthiolane 1-oxide binding to *Eq*ADH (dotted Phe-93) or E/F93W enzyme. The E/F93W structure with NAD^+ and trifluoroethanol was determined from 23 430 reflections, from 8.0 to 2.5 Å resolution, refined to an R_{value} of 21.5% (R_{free} 27.3%) for a triclinic crystal with unit cell dimensions of $a = 44.15$ Å, $b = 51.20$ Å, $c = 92.52$ Å, $\alpha = 92.3°$, $\beta = 102.9°$, and $\gamma = 109.7°$ (S. Selvaraj and S. Ramaswamy, unpublished).

empty space created by the F93A substitution (Figure 2B). In contrast to small sulfoxides or phenyl methyl sulfoxide, the long alkyl side chains of BTO and 3-hexylthiolane 1-oxide could not be accommodated in the space near Ala-93, which explains why BTO and 3-hexylthiolane 1-oxide are relatively weaker inhibitors for E/F93A enzyme.

The E/F93W and wild-type enzymes have similar affinities for most inhibitors. The three-dimensional structure of the E/F93W enzyme, complexed with NAD^+ and trifluoroethanol, shows that the indole ring of Trp-93 occupies the position of the benzene ring in the wild-type enzyme and does not noticeably change the local structure (Figure 3C). Modeling of dimethyl sulfoxide or thiolane 1-oxide into the active site of the mutated enzyme suggests that the substitution would not produce unfavorable steric interactions or altered positioning (Figure 3C). The F93W substitution does not appear to alter binding interactions significantly.

Stereoselectivity. The *S* isomers of various sulfoxides bind better than the corresponding *R* isomers to *Eq*ADH. The *S* enantiomers of hexyl methyl sulfoxide and phenyl methyl sulfoxide bind 5−8-fold tighter to *Eq*ADH than do their respective *R* enantiomers (Table 4). The 1*S* isomers of BTO

Table 3: Inhibition of Horse Liver Alcohol Dehydrogenases by Sulfoxides[a]

inhibitor	K_i, μM			
	*Eq*ADH	E/F93W	E/F93A	E/S48T
dimethyl sulfoxide	1500	970	1200	28000
ethyl methyl sulfoxide	410	890	570	6000
phenyl methyl sulfoxide	58[b]	440	44[b]	17000
hexyl methyl sulfoxide	31[b]	160	470	410
thiolane 1-oxide	19[c]	16	21	2100
3-butylthiolane 1-oxide	0.56	1.0	12	39
3-hexylthiolane 1-oxide	0.19[c]	0.45	1.5	5.1

[a] In 46 mM sodium phosphate buffer, pH 7, at 25 °C. [b] Calculated from the values in Table 4 using the relationship $2/K_{i(racemate)} = 1/K_{i(S)} + 1/K_{i(R)}$. [c] From refs 1 and 2.

also bind 10−100-fold tighter than their corresponding 1*R* isomers (*7*). With the sulfoxide oxygen bound to the zinc and the lone pair electrons oriented toward Phe-93, the alkyl chain of the (*S*)-sulfoxide fits better into the active site than does the *R* isomer, which results in a lower K_i for the *S* isomer. However, phenyl methyl sulfoxide shows the opposite stereoselectivity for E/F93A (20-fold reversal compared to *Eq*ADH). *Hs*ADH α (Thr-48, Ala-93) has less stereoselectivity for hexyl methyl sulfoxide but more for phenyl methyl sulfoxide than E/F93A does. *Hs*ADH β_1 (Thr-48, Phe-93) is selective for the *R* isomer of phenyl methyl sulfoxide. Thus, Phe-93 and Ser-48 tend to determine selectivity for the *S* isomers, whereas Ala-93 and Thr-48 interact better with the *R* isomers.

Cation−π Interaction and Biochemical Studies. Three-dimensional structures of *Eq*ADH−NADH complexed with BTO or dimethyl sulfoxide suggest that there is a cation−π interaction between the electron-rich π cloud of Phe-93 and the positively charged sulfur (*7*, *32*, *33*). The distance between the sulfoxide sulfur and the plane of the benzene ring is 3.4−3.7 Å, and the Mulliken charge on the sulfur is about +0.9. Binding constants for "guest" sulfoxide, sulfonium, or quaternary ammonium ions to a "host" (aromatic molecule) are comparable (*34*). The typical distance for "amino−aromatic" interactions is 3.4−6 Å according to the survey of Burley and Petsko (*35*). Values below 3.4 Å are rarely observed because of unfavorable van der Waals contacts. The optimum distance for cation−quadrupole interaction between K^+ and benzene is about 2.7 Å based on electrostatic calculations (*36*). However, the stabilization energy of K^+−benzene at 3.4−4.4 Å distance is still about −10 kcal/mol, since it decreases relatively slowly with increasing ion−ligand distance, and this prediction is in fairly good agreement with Δ*H* values from gas-phase experiments.

We did biochemical and computational studies to evaluate the cation−π interaction between the sulfoxide and Phe-93. Unfortunately, no amino acid substitution would eliminate only the quadrupole moment from Phe-93 without affecting the binding interactions, but substitution with Trp could strengthen the cation−π interaction. The *ab initio* calculation shows that the interaction energy (Δ*G*) between Na^+−indole (32.6 kcal/mol) is higher than that between Na^+−benzene (27.1 kcal/mol) in the gas phase (*37*). Since the F93W substitution apparently does not disturb the structure (Figure 3C), an intensified cation−π interaction might be observed. However, neither the F93W nor F93A substitution significantly alters the binding affinities of small sulfoxides (Table 3). For comparison, substitution of aromatic residues (especially Trp-86) at the active site of acetylcholine esterase by aliphatic side chains substantially decreased binding affinities for cationic inhibitors (*38*).

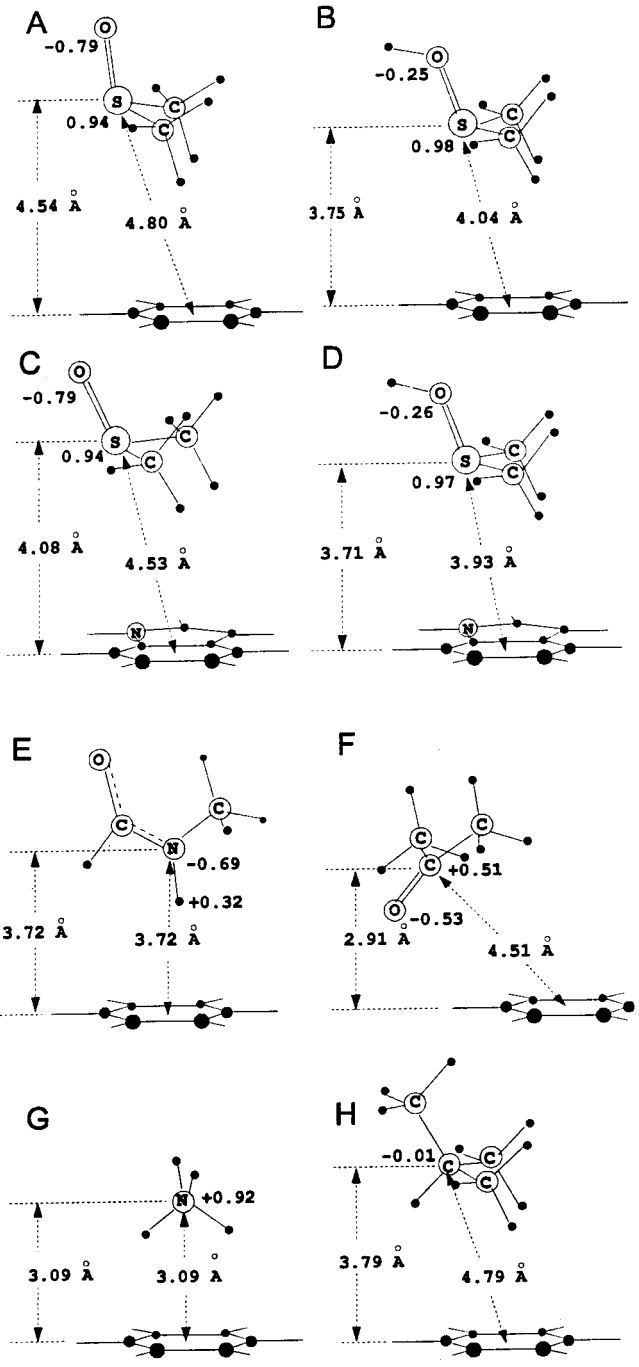

FIGURE 3: Complexes of various compounds optimized with HF/6-31G**. The distances from the sulfur, carbonyl carbon, tertiary carbon, or nitrogen to the plane and to the center of the benzene or six-membered ring of indole and Mulliken charges are given: (A) dimethyl sulfoxide−benzene, (B) protonated dimethyl sulfoxide−benzene, (C) dimethyl sulfoxide−indole, (D) protonated dimethyl sulfoxide−indole, (E) *cis*-*N*-methylformamide−benzene, (F) acetone−benzene, (G) NH_4^+−benzene, (H) 2-methylpropane−benzene.

Evidence for a cation−π interaction in binding of amides was also sought by comparing the inhibition of *Eq*ADH variants by cyclic sulfoxides and cyclic amides (lactams) with five- or six-membered rings (Table 5). Amides could form a cation−π interaction with Phe-93 (*31*). The lactams bind to the E−NADH complex, as they are uncompetitive

Table 4: Stereoselective Inhibition of ADHs by Sulfoxides[a]

inhibitor	isomer	K_i, μM *Eq*ADH	E/F93A	*Hs*-α	*Hs*-β_1
hexyl methyl sulfoxide	*S*	19	ND[b]	460	ND
	R	94	ND	810[c]	ND
phenyl methyl sulfoxide	*S*	33	73	180	1800
	R	270	32	40	1100

[a] In 46 mM sodium phosphate buffer, pH 7, at 25 °C. [b] ND, not determined. [c] Calculated from the value in Table 2 and the relationship $2/K_{i(\text{racemate})} = 1/K_{i(S)} + 1/K_{i(R)}$.

Table 5: Inhibition of Horse Liver Alcohol Dehydrogenases by Cyclic Sulfoxides and Lactams[a]

inhibitor	K_i, μM *Eq*ADH	E/F93W	E/F93A
δ-valerolactam	1650[b]	78000	7.3
2-pyrrolidinone	6300	5200	50
thiane 1-oxide	480[c]	1800	110
thiolane 1-oxide	19[c]	16	21

[a] In 46 mM sodium phosphate buffer, pH 7, at 25 °C. [b] From ref 31. [c] From ref 1.

inhibitors against varied ethanol concentrations and competitive against carbonyl substrates. Lactams have less positive charge on the carbonyl carbon and nitrogen than the sulfur of the sulfoxides has and are relatively poor inhibitors for wild-type *Eq*ADH. The F93W mutation does not change binding constants for the inhibitors with five-membered rings but decreases by severalfold binding of the inhibitors with six-membered rings. The F93A substitution increases by 100-fold the binding of 2-pyrrolidinone and δ-valerolactam, apparently by relieving steric hindrance, but thiolane 1-oxide binding and thiane 1-oxide binding are much less affected. The lactams and sulfoxides probably bind in different orientations. The inhibition studies with *Eq*ADH and the mutated enzymes provide no evidence to support a strong contribution of the cation−π interaction to the binding of sulfoxides or lactams to *Eq*ADH.

Computational Studies on Cation−π Interactions. The potential contributions of cation−π interactions between sulfoxides and the electron-rich π cloud of Phe-93 or Trp-93 were also evaluated with *ab initio* calculations in the gas phase (Table 6). Optimization of the dimethyl sulfoxide−benzene (or indole) complexes starting from the geometry determined by X-ray crystallography moves the sulfur away from the plane of the benzene ring (to 4.5 Å or to 4.1 Å from the indole ring), while the two methyl groups of dimethyl sulfoxide make van der Waals contact with the benzene or indole ring (Figure 3A,C). The interaction energy of dimethyl sulfoxide with benzene or indole is much lower (−4 kcal/mol or less) than that of typical cation−π interactions (more than −10 kcal/mol), even considering that ΔG is underestimated (*34*, *39*, *40*). After correction with density functional theory, the calculated complexation energy of NH_4^+ with benzene is slightly smaller than the experimental ΔG (about −19 kcal/mol, ref *41*), but this correction decreases the calculated interaction energies of dimethyl sulfoxide with benzene or indole.

Interactions were also evaluated with compounds similar to dimethyl sulfoxide. With acetone or *cis*-*N*-methylformamide, the distances from the central atoms (carbonyl carbon or amide nitrogen) to the benzene plane are less than 4 Å, and the complexation energies are comparable to that of the dimethyl sulfoxide−benzene complex even though the central atoms have much less charge (Figure 3E,F). If we assume that the interaction of 2-methylpropane with benzene (Figure 3H) reflects the van der Waals interaction of dimethyl sulfoxide with benzene, the cation−π interaction could contribute about −1 kcal/mol for dimethyl sulfoxide−benzene and −2 kcal/mol for dimethyl sulfoxide−indole complexes. These magnitudes are comparable to the energies of interaction for the NH moieties of imidazole and carboxyamide groups with aromatic rings (Table 6, ref *10*). The calculations suggest that the binding of sulfoxide with indole should be about 5-fold better than with benzene, but this effect was not observed with the F93W mutant enzyme.

Table 6: Complexation Energies between Sulfoxides or Other Compounds and Aromatic Molecules after Optimization with HF/6-31G**[a]

complex	ΔE, kcal/mol HF	DFT/B3LYP[b]	DFT/B3PW91[c]
NH_4^+−benzene	−15.3	−17.4	−16.9
$(CH_3)_2SO$−benzene	−1.85	−1.66	−1.18
$(CH_3)_2SO$−indole	−2.70	−2.52	−1.84
cis-CH_3NHCHO−benzene	−2.64	−2.66	−2.22
$(CH_3)_2CO$−benzene	−1.72	−1.65	−1.04
$(CH_3)_3CH$−benzene	−0.59	−0.49	−0.082
$(CH_3)_2SOH^+$−benzene	−8.18	−8.68	−7.97
$(CH_3)_2SOH^+$−indole	−11.9	−12.8	−12.1

[a] Convergence of Hartree−Fock optimization was set to 10^{-8} Hartree. [b] Correlation and exchange energy correction by density functional theory (DFT) was made after HF optimization with the 6-31G** basis set. B3 for exchange energy calculation (*23*) and LYP for correlation energy calculation (*24*). [c] B3 for exchange energy calculation and PW91 for correlation energy calculation (*25*).

In the observed *Eq*ADH−NADH−sulfoxide complexes, the distance between the sulfur and the benzene ring of Phe-93 is about 3.3 Å, shorter than the 4.5 Å in the optimized model complex. The energetically unfavorable steric effect must be offset by other interactions. The cation−π interaction between the sulfur and benzene might be augmented due to polarization of the sulfoxide bound to the catalytic zinc. A model for the maximum effect of polarization is the protonated sulfoxide. The calculations show that the sulfur remains 3.7−4.0 Å away from the planes of benzene or indole with 8−13 kcal/mol stabilization energy (Table 6 and Figure 3B,D). In the enzyme, however, the zinc is neutralized by two thiolates furnished by cysteine residues and should have much less effect on the sulfoxide. We conclude that the contribution of the cation−π interaction to the binding of sulfoxides to ADH is relatively small. It appears that good van der Waals interactions account for the tight binding of thiolane 1-oxides.

Pharmacological Implications of Inhibition of ADH by Sulfoxides. *Hs*ADH β and γ show higher catalytic activity than α, π, and σ enzymes for methanol and ethanol (Table 7). It is interesting that *Hs*ADH γ is also active on steroid substrates (*42*). *Hs*ADH β and γ contribute significantly to the total ADH activity in human liver (*43*, *44*). We studied the γ_2 enzyme, but the γ_1 enzyme would probably show similar kinetic and inhibition patterns since γ_1 and γ_2 are identical except at residues 271 (γ_1-Arg and γ_2-Gln) and 349 (γ_1-Ile and γ_2-Val), which are far from the substrate binding

Table 7: Kinetic Parameters of Human Alcohol Dehydrogenases[a]

	ethanol			methanol		
*Hs*ADH	K_m (mM)	V_{max} (min^{-1})	V_{max}/K_m (mM^{-1} min^{-1})	K_m (mM)	V_{max} (min^{-1})	V_{max}/K_m (mM^{-1} min^{-1})
α[b]	4.2	27	6.4	380	0.43	0.0011
β_1[c]	0.0022	3.3	160	9.9	5.8	0.59
γ_2	0.21	32	150	18	4.0	0.23
π[d]	120	470	4		NA	
σ[e]	28	1800	65	4000	51	0.013

[a] The kinetics for methanol oxidation was determined with 2.4 mM NAD^+ in 46 mM sodium phosphate buffer with 7.7 mM semicarbazide and 0.25 mM EDTA, pH 7.5, at 25 °C. Standard errors of fits with HYPER (*20*) were 5−15%. [b] Data for ethanol were determined at pH 7.5, 25 °C (*48*), and for methanol with the monkey α enzyme at pH 7.5, 25 °C (*30*). [c] Data for ethanol, pH 7.5, 25 °C (*49*). [d] Data for ethanol, pH 10, 25 °C (*50*). *Hs*ADH π is not active (NA) on methanol (*47*). [e] Data for ethanol, pH 7.4, 25 °C (*13*).

site (*14*). The sulfoxides, especially BTO, are selective inhibitors of *Hs*ADH γ_2 enzyme and might be useful *in vivo*. BTO is an effective inhibitor of ethanol metabolism in rats and has low toxicity (*2*). In contrast, *Hs*ADH β is not strongly inhibited by BTO, and other types of inhibitors may be required for this enzyme.

ACKNOWLEDGMENT

We thank Dr. Thomas D. Hurley at the Indiana University Medical School, Indianapolis, and Dr. Jan-Olov Höög, Karolinska Institutet, Stockholm, for expression vectors for human ADHs; Drs. S. Selveraj and S. Ramaswamy, Swedish University of Agricultural Sciences, Uppsala, for determining the structure of the *Eq*ADH/F93W enzyme; Dr. Daniel M. Quinn, The University of Iowa, Iowa City, for comments on Gaussian calculations; and in our laboratory, Dr. John F. Schindler and Kristine B. Berst for purification and characterization of human ADHs and Dr. Keehyuk Kim for vectors for mutated horse liver ADHs.

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BI9727040