INHIBITION OF RAT LIVER GLUTATHIONE *S*-TRANSFERASE ACTIVITY BY APROTIC SOLVENTS

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1. Introduction

Conjugation with glutathione is a major detoxication pathway for many alkene and arene oxides, as well as a variety of other electrophilic compounds [1–3]. The significance of this pathway for epoxide metabolism is accentuated by the findings that the competing epoxide hydrase pathway is involved in the enzymatic synthesis of what may be the ultimate carcinogen from benzo[a]pyrene [4] and other polycyclic aromatic hydrocarbons, and that there is relatively much higher in vitro activity of glutathione *S*-transferases than epoxide hydrase in several mammalian tissues [5].

A variety of aprotic solvents have been employed as vehicles for the lipophilic substrates of the glutathione *S*-transferases, including acetone [6], acetonitrile [1,2,5,7–11], dimethyl sulfoxide [2,6,12–14], ethanol [12–20], methanol [1,21] and tetrahydrofuran [1,2,10,11]. However, little attention has been paid to the effects of these solvents on the enzymes studied, even in highly sophisticated kinetic analyses. We have shown that commonly used solvents have profound, substrate-dependent effects on various monooxygenase reactions in vitro [22].

We demonstrate here the inhibitory effect of 12 aprotic solvents on styrene oxide conjugation with glutathione catalyzed by the microsomal supernatant fraction of rat liver. Inhibition is most pronounced at low substrate concentrations, but statistically significant even at high substrate concentrations. The enzymatic conjugation of 1,2-dichloro-4-nitrobenzene with glutathione is inhibited by ethanol, methanol, acetonitrile and tetrahydrothiophene dioxide, and that of benzo[a]pyrene 4,5-oxide by tetrahydrofuran, dioxane and tetrahydrothiophene dioxide.

2. Experimental

The soluble fraction (176 000 × *g* supernatant) was prepared from livers of adult male specific pathogen-free Sprague-Dawley rats as in [5] and was used as the enzyme source after dilution to 1–3 mg protein/ml. Protein content of these preparations was determined by the Lowry method [23] and bovine serum albumin was used as the reference standard. The rate of enzyme-catalyzed conjugation of styrene oxide and benzo[a]pyrene 4,5-oxide was determined essentially as in [5]. [^{14}C]Styrene oxide (final conc. 50 μM or 1 mM as indicated) was added in 1 μl acetonitrile and [^{3}H]benzo[a]pyrene 4,5-oxide (4,5-BPO) in 1 μl dimethyl sulfoxide in 0.9 ml total incubation vol. To correct for nonenzymic conjugation, a control was run without tissue; the effect of tissue on carryover of radioactivity was determined by running nonincubated controls with and without added tissue. The incubation was stopped by adding 2.4 ml ethyl acetate followed by immediate thorough mixing. The formed dihydrodiols and phenols were removed along with the unreacted oxides by 3 extractions with ethyl acetate. The radioactivity in the remaining aqueous phase, representing solely the conjugate [2], was counted. The incubations were carried out in triplicate. Conjugation of 1,2-dichloro-4-

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nitrobenzene was followed at 350 nm in a Beckman Acta III spectrophotometer using a nonenzymic reaction mixture as the reference [24]. The total reaction volume was 3 ml and the final concentration of the substrate was 50 μM added in 10 μl dimethyl sulfoxide. With all 3 substrates, the incubation mixture was buffered with 0.133 M Hepes–NaOH buffer (pH 7.85) and the concentration of glutathione was 3.5 mM. The initial linear reaction velocity was determined.

3. Results and discussion

The main emphasis of this study was to investigate the effects of various organic solvents commonly used as vehicles for substrates in assays for glutathione *S*-transferase activity. Therefore, the solvents were compared on a v/v basis and not on a molar concentration basis. The results are shown in table 1.

All organic solvents studied effectively inhibited

Table 1
Effect of organic solvents on the glutathione *S*-transferase activity of rat liver soluble fraction with styrene oxide, benzo[a]pyrene 4,5-oxide or 1,2-dichloro-4-nitrobenzene

Solvent	Concentration % (v/v)	mol/l	Glutathione *S*-transferase activity[a]		
			Styrene oxide	Benzo[a]pyrene 4,5-oxide	1,2-Dichloro-4-nitrobenzene
Acetone	3.3	0.45	47.2 ± 3.9[b]	89.4 ± 5.5	80.1 ± 4.5
	0.56	0.075	85.7 ± 6.7		
Acetonitrile	3.3	0.63	79.0 ± 5.2[c]	92.6 ± 3.0	59.2 ± 1.4
	3.3	0.63	48.8 ± 4.5		
	0.56	0.11	83.8 ± 3.4		
	0.11	0.021	95.4 ± 5.7		
1,2-Dimethoxyethane	3.3	0.32	51.2 ± 11.4	80.4 ± 6.8	103 ± 0.9
	0.56	0.054	73.0 ± 9.0		
Dimethylformamide	3.3	0.43	61.4 ± 4.7	91.7 ± 6.2	84.6 ± 2.5
	0.56	0.072	93.4 ± 3.6		
Dimethyl sulfoxide	3.3	0.47	74.5 ± 4.4	85.9 ± 5.5	85.8 ± 3.9
	0.56	0.078	95.9 ± 5.5		
Dioxane	3.3	0.39	45.5 ± 8.6	59.0 ± 6.9	47.3 ± 0.6
	0.56	0.065	77.0 ± 13.1		
1,3-Dioxolane	3.3	0.47	47.1 ± 8.7	87.7 ± 5.4	90.1 ± 10.4
	0.56	0.079	81.0 ± 6.8		
Ethanol	3.3	0.57	43.0 ± 5.7	83.9 ± 4.9	62.9 ± 0.1
	0.56	0.095	83.6 ± 3.8		
Methanol	3.3	0.82	57.9 ± 3.7	85.1 ± 4.7	60.6 ± 1.9
	0.56	0.14	88.5 ± 2.5		
Bis-(2-Methoxyethyl)-ether	3.3	0.23	60.1 ± 10.6	78.6 ± 3.1	112 ± 15.1
	0.56	0.039	82.8 ± 7.1		
Tetrahydrofuran	3.3	0.41	25.7 ± 9.6	60.7 ± 14.0	93.5 ± 7.2
	0.33	0.041	75.9 ± 6.5	81.3 ± 7.2	
	0.11	0.014	81.6 ± 4.4	93.5 ± 3.3	
Tetrahydrothiophene 1,1-dioxide	3.3	0.35	38.7 ± 4.8	44.0 ± 6.2	60.4 ± 5.2

[a] Expressed as % control activity (47.1 ± 13.4, 55.4 ± 11.2, and 9.72 ± 0.79 nmol/min/mg protein[b] for styrene oxide, benzo[a]pyrene 4,5-oxide and 1,2-dichloro-4-nitrobenzene, respectively). The solvents and solvent concentrations used in the control incubation mixtures were 0.11% (v/v) acetonitrile for styrene oxide, 0.11% (v/v) dimethyl sulfoxide for benzo[a]pyrene 4,5-oxide, and 0.33% (v/v) dimethyl sulfoxide for 1,2-dichlorobenzene. Final glutathione concentration was 3.5 mM for each substrate. The substrate concentrations were 50 μM, 10 μM and 50 μM for styrene oxide, benzo[a]pyrene 4,5-oxide, and 1,2-dichloro-4-nitrobenzene, respectively

[b] Mean ± SD (n = 4 rats)

[c] Styrene oxide was 1 mM

the enzymatic conjugation of styrene oxide with glutathione. However, when 1 mM styrene oxide was employed as the substrate, the inhibition was considerably less pronounced although it was still statistically significant. Acetonitrile (2.6% v/v) was reported [9] not to affect styrene oxide conjugation by Wistar rat supernatant function when 1 mM substrate was used. The substrate in this study was added in 1 μl (0.1%, v/v) acetonitrile. Doubling this concentration of solvent did not significantly alter the observed enzyme activity. Thus, it seems possible to use 0.1% acetonitrile as the vehicle for styrene oxide even at low substrate concentrations (50 μM), which are required in kinetic studies. Of the solvents tested, the most effective inhibitor of styrene oxide conjugation was tetrahydrofuran. Concentrations as low as 0.11% still caused a statistically significant decrease of enzyme activity (~20%). Distillation of tetrahydrofuran (to remove the butylated hydroxytoluene used as preservative) immediately prior to use did not affect enzyme inhibition.

Benzo[a]pyrene 4,5-oxide has very limited solubility in water [10]. Therefore, it was studied only at 10 μM. The solvents investigated inhibited its conjugation much less than that of either styrene oxide or 1,2-dichloro-4-nitrobenzene. Tetrahydrofuran, a good solvent for the storage of arene oxides at reduced temperatures [25,26], was a potent inhibitor, as were dioxane and tetrahydrothiophene dioxide. Dimethylformamide and acetone, even at high concentrations, did not cause any significant enzyme inhibition with this arene oxide substrate. The conspicuously higher affinity of polycyclic arene oxides for glutathione *S*-transferases [2,10,11] may explain the relative lack of solvent effects on the enzymic conjugation of benzo[a]pyrene 4,5-oxide.

1,2-Dichloro-4-nitrobenzene is widely used as a substrate for glutathione *S*-transferase activity. The enzymatic conjugation of this substrate with glutathione was intermediate between that of styrene oxide and benzo[a]pyrene 4,5-oxide with respect to solvent sensitivity; of the 12 solvents tested, 5 were effective inhibitors.

Conspicuous differences exist among the different substrates in their sensitivity toward different solvents: acetonitrile is a potent inhibitor of styrene oxide and 1,2-dichloro-4-nitrobenzene conjugation, but does not affect conjugation of benzo[a]pyrene 4,5-oxide. Tetrahydrofuran is the most potent inhibitor of arene oxide conjugation, but has no effect on 1,2-dichloro-4-nitrobenzene conjugation. Methanol and ethanol are among the most potent inhibitors of 1,2-dichloro-4-nitrobenzene conjugation but have no marked effect on either of the other two substrates. The multiplicity of glutathione *S*-transferases in rat liver supernatant fraction [15,16], with overlapping specificities and wide variations in substrate affinities [10,11,15,16], may account for the pattern of solvent inhibition of different substrates demonstrated here. With any substrate and enzyme source combination, it seems advisable to first check the effect of the solvents to be used on the conjugation rate, particularly if kinetic studies are anticipated.

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