ETHANOL OXIDATION IN THE MICROSOMAL FRACTION OF RAT LIVER

Mary K. Roach, W.N. Reese, Jr. and P.J. Creaven
Texas Research Institute of Mental Sciences
Texas Medical Center Houston, Texas 77025

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Summary

The NADPH dependent oxidation of ethanol by the liver microsomal fraction has been investigated. NADPH cannot be replaced by H_2O_2 but can be replaced by glucose + glucose oxidase in the system. Aminotriazole inhibits the activity but less than it does catalase. It is concluded that ethanol oxidation by the microsomal fraction is mediated through H_2O_2 dependent systems one of which is catalase.

The oxidation of ethanol to acetaldehyde by the microsomal fraction of liver has been reported by Orme-Johnson and Ziegler (1) and Lieber and DeCarli (2). The reaction is NADPH and oxygen dependent, is inhibited by carbon monoxide and was considered by both groups of workers to be mediated through the mixed function oxidase system responsible for the oxidation of non-polar compounds. The activity of the system is increased by ethanol consumption (2) and by pretreatment with phenobarbital and benz(a)pyrene (3). However, certain properties of the system, namely its inhibition by cyanide and azide and its lack of inhibition by 2-diethylamino-ethyl-2,2-diphenylvalerate (SKF 525-A) suggest that the activity may not be due to the microsomal mixed function oxidase system. We here report the results of studies into the nature of the ethanol oxidizing activity of the microsomal fraction of liver carried out in an attempt to elucidate the nature of the enzymic process involved.

Materials and Methods

Glucose oxidase was obtained from Calbiochem. Bovine liver catalase was obtained from Sigma Chemical Co. 2-Diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A) was kindly supplied by Smith, Kline and French. 3-Amino-1,2,4-triazole was obtained from Aldrich Chemical Co. 4-Ethoxybiphenyl was prepared and purified as previously described (4).

Male Sprague-Dawley rats, 50 g, were used. They were maintained on Wayne Lab Blox and water ad libitum. Animals pretreated with amino-triazole received 2 g/Kg in 0.5 ml isotonic saline intraperitoneally 1 hr. before being sacrificed. Preparation of the microsomal fraction of liver was as previously described (5).

Microsomes (≡ 0.25g liver) were incubated in a final volume of 3 ml with 66 mM ethanol, 1 mM NADPH, 33 mM phosphate buffer, pH 7.4, for 30 min. with shaking at 37°C in stoppered 10 ml serum vials. Acetaldehyde was assayed by the gas chromatographic method previously described (6). In experiments in which a H_2O_2 generating system was substituted for NADPH, 1.48 mM glucose and 0.4 µg glucose oxidase were added to the incubation mixture and NADPH was omitted. Catalase was assayed by the perborate method of Feinstein (7) and the dealkylation of 4-ethoxybiphenyl as previously described (4).

Results

Ethanol incubated with a microsomal preparation as described under methods is oxidized to acetaldehyde. This activity is NADPH and O_2 dependent and is inhibited by carbon monoxide but is also inhibited by CN^- and N_3^- and is unaffected by SKF 525-A (Table 1). NADPH cannot be replaced by H_2O_2 but a system in which a H_2O_2 generating system (glucose + glucose oxidase) is substituted for NADPH is fully

TABLE 1

Comparison of Ethanol Oxidation with 4-Ethoxybiphenyl O-Dealkylation by the Microsomal Fraction of Rat Liver

	Ethanol Oxidation % of Control	O-Dealkylation % of Control
Control (Complete system)	100	100
Minus NADPH	0	0
Minus NADPH plus H_2O_2 (5×10^{-4}M)	5	0
Minus NADPH plus H_2O_2 (3×10^{-2}M)	5	0
Minus NADPH plus glucose + glucose oxidase	100	0
Inhibitors		
Carbon monoxide ($CO:O_2$=2.25:1 CO=9%)	55	60
SKF 525-A (4×10^{-4}M)	100	55
Cytochrome c (4×10^{-5}M)	36	75
Sodium cyanide (2×10^{-3}M)	16	100
Sodium azide (2×10^{-3}M)	16	100

active (Table 1). Repeated washing of the microsomal fraction reduces the activity to low levels but does not abolish it; microsomal catalase follows a similar pattern (Table 2). Pretreatment with aminotriazole inhibits ethanol oxidation less than 50% and microsomal catalase more than 80% (Table 3).

Discussion

The ethanol oxidizing activity of the liver microsomal fraction has been considered to be due to the microsomal mixed function oxidase system (1,2). However, the microsomal fraction contains an NADPH

TABLE 2

Effect of Washing on the Ethanol Oxidizing and Catalase Activities of Liver Microsomes

No. of Washes	0	1	2	3	4
Systems:					
Microsomes + NADPH (Acetaldehyde formed µg/g liver/min)*	6.89 ± 0.98	4.99 ± 0.97	4.89 ± 1.03	4.45 ± 1.53	3.94 ± 1.42
Microsomes + Glucose + Glucose Oxidase (Acetaldehyde formed µg/g liver/min)*	8.80 ± 1.26	4.90 ± 0.13	4.12 ± 0.69	3.96 ± 1.19	4.09 ± 1.27
Catalase Activity (mEq perborate destroyed/g liver/min)	7.71	1.70	0.86	0.73	0.62

*Figures are the mean of 3 experiments ± S.D.

oxidase which generates peroxide (8) and this with residual catalase could lead to the oxidation of ethanol. The possibility that residual catalase activity is responsible for the ethanol oxidizing activity of the microsomal fraction was discounted by Orme-Johnson and Ziegler (1) on the grounds of pyridine nucleotide specificity and the failure of added hydrogen peroxide to replace the activity of NADPH in the system. The subcellular distribution of the activity and its relative insensitivity to inhibition by cyanide led Lieber and DeCarli (2) also to discount the possibility that catalase is the effective agent. Moreover, the system described by Gillette, et al. (8) did not oxidize methanol to formaldehyde in the absence of added catalase. However, the behavior of the microsomal ethanol oxidizing activity toward inhibitors differs markedly from that of the dealkylation of 4-ethoxybiphenyl, a typical microsomal mixed

TABLE 3

Inhibition by Aminotriazole of Catalase Activity and Ethanol Oxidation

Enzyme	Microsomal Fraction Inhibition %		Bovine Liver Catalase** Inhibition %
	Expt. 1	Expt. 2	
Catalase activity*	88	84	94
NADPH dependent ethanol oxidation	40	49	--
Glucose + glucose oxidase dependent ethanol oxidation	49	48	94

*Measured by the perborate assay -- see under methods.

**Dialyzed against aminotriazole and H_2O_2 by the procedure of Margoliash, et al. (9).

function oxidase (4)(Table 1). NADPH cannot be replaced by added hydrogen peroxide in the oxidation of ethanol in agreement with the results of Orme-Johnson and Ziegler (1) but can be replaced by a hydrogen peroxide generating system such as glucose + glucose oxidase, indicating that the oxidation of ethanol by the microsomal fraction is at least partly due to NADPH oxidase and residual catalase which cannot be removed even by repeated washing (Table 2).

Aminotriazole which is reported to produce irreversible, specific inhibition of catalase (9) inhibits microsomal catalase activity 85-90% but inhibits the NADPH and the glucose + glucose oxidase dependent microsomal ethanol oxidizing activities only 40-50% (Table 3). Somewhat similar results with methanol oxidation were reported by Tephley, et al. (10) who found that aminotriazole inhibited catalase 94% and methanol oxidation only 70%.

Catalase is also inhibited by cyanide. Microsomal ethanol oxidation was reported to be inhibited 17% by 10^{-4}M CN^- (2) and 68% by 10^{-3}M CN^- (1). We have found 84% inhibition by 2×10^{-3}M CN^- of microsomes washed only once. However, inhibition of rigorously washed microsomes by 2×10^{-4}M CN^- varied with different microsomal preparations from 27% to 57%. The reason for these discrepancies is not presently clear.

The results presented here indicate that the system responsible for the microsomal oxidation of ethanol is H_2O_2 dependent. The results with aminotriazole inhibition suggest that about 60% of the activity of the system is due to catalase and about 40% to some other system. The contribution, if any, of the microsomal mixed function oxidase system to the oxidation of ethanol would appear to be insignificant.

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