Hepatic Microsomal Alcohol-oxidizing System

AFFINITY FOR METHANOL, ETHANOL, PROPANOL, AND BUTANOL*

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Oxidation of methanol, ethanol, propanol, and butanol by the microsomal fraction of rat liver homogenate is described. This microsomal alcohol-oxidizing system is dependent on NADPH and molecular oxygen and is partially inhibited by CO, features which are common for microsomal drug-metabolizing enzymes. The activity of the microsomal alcohol-oxidizing system could be dissociated from the alcohol peroxidation via catalase-H$_2$O$_2$ by differences in substrate specificity, since higher aliphatic alcohols react only with the microsomal system, but not with catalase-H$_2$O$_2$. Following solubilization of microsomes by ultrasonication and treatment with deoxycholate, the activity of the microsomal alcohol-oxidizing system was separated from contaminating catalase by DEAE-cellulose column chromatography, ruling out an obligatory involvement of catalase-H$_2$O$_2$ in the activity of the NADPH-dependent microsomal alcohol-oxidizing system. In intact hepatic microsomes, the catalase inhibitor sodium azide slightly decreased the oxidation of methanol and ethanol, but not that of propanol and butanol, indicating a facultative role of contaminating catalase in the microsomal oxidation of lower aliphatic alcohols only. It is suggested that the microsomal alcohol-oxidizing system accounts, at least in part, for that fraction of hepatic alcohol metabolism which is independent of the pathway involving alcohol dehydrogenase activity.

The capacity of hepatic microsomes to oxidize ethanol to acetaldehyde has been well established in a variety of studies (2–6). This microsomal ethanol-oxidizing system requires NADPH and molecular oxygen and is partially inhibited by CO (3), sharing thereby properties with other microsomal drug oxidations. A major part of the ethanol-oxidation activity in microsomes was ascribed to a catalase-independent pathway (3–6). This was substantiated subsequently by solubilization and isolation of MEOS* using DEAE-cellulose column chromatography (7–9). More recently, MEOS was also dissociated from NADPH oxidase activity itself (10) the enzyme which generates H$_2$O$_2$ in the microsomal fraction (11).

However, microsomes contain catalase as a contaminant (3). Since ethanol is also a substrate for catalase (12), some groups have attributed the activity of the microsomal ethanol-oxidizing system exclusively to a process involving catalase (13, 14) in which the rate of H$_2$O$_2$ generation is thought to be the rate-limiting step (14). Conversely, alcohols with longer aliphatic chains are extremely poor or virtually not at all substrates for catalase-H$_2$O$_2$ (12, 15). It was therefore of interest to study the activity of the microsomal alcohol-oxidizing system (MEOS) with respect to various alcohols to possibly distinguish the microsomal system from the catalase-mediated alcohol oxidation by differences in substrate specificity. Parts of this study were published in preliminary form (16, 17).

EXPERIMENTAL PROCEDURES

Materials—The chemicals and enzymes were obtained from the following sources: NADPH (type I), NADP$^+$ (yeast), α-NADH (grade II), β NADH, β NAD$^+$ (grade III), D,L-isocitrate (type I), isocitric dehydrogenase, glutathione (reduced form), DEAE-cellulose (medium mesh), Trizma (2-amino-2-hydroxymethyl-1,3-propanediol, Tris, pH 7.7 at 4°), dithiothreitol, μ-chloromercuri-benzoic acid, and cytochrome c (type VI) from Sigma Chemical Co., St. Louis, Mo.; disodium-EhTA, sodium azide, D-glucose, hydrogenone, methanol, 1-propanol, 1-butanol, formaldehyde, sodium formate, urea, semicarbazide hydrochloride, perchloric acid, and trichloroacetic acid from Fisher Scientific Co., Fair Lawn, N.J.; sodium cholate and sodium deoxycholate from Schwarz-Mann Co., Orangeburg, N.Y.; pyrazole, acetaldelyde, propionaldehyde, butyraldehyde, uric acid, and d-a-tocopherol from Eastman Kodak Co., Rochester, N.Y.; glucose oxidase (type D from Boehringer Mannheim Corp., New York, N.Y.); ethanol (dehydrated) from U.S. Industrial Chemicals Co., Tuscola, Ill.; malondialdehyde bis(diethylacetal) from Schuchardt Co., Munich, Germany, and the gas mixtures from Matheson Gas Products, East Rutherford, N.J. Preparation of Microsomes—Male Sprague-Dawley rats (strain CD, Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 280 to 380 g were used. They were fed Purina laboratory chow ad libitum and had free access to tap water. The rats were killed by decapitation, their livers perfused with ice-cold 0.15 M KCl through the...
portal vein, excised, chilled, and homogenized in 3 volumes of 0.15 M KCl. The following steps were carried out at 0–4°C. The 25% homogene-
nate was centrifuged at 10,000 x g for 30 min followed by centrifuga-
tion of the supernatant at 105,000 x g for 30 min. The resulting pellet
was resuspended in 0.15 M KCl, and washed microsomes were obtained
by spinning this suspension at 105,000 x g for another 30 min. The
microsomes were used either the same day, or the pellets were covered
with 0.1 M phosphate buffer (pH 7.4) and stored overnight under
nitrogen at −20°C. Microsomal protein concentration was determined
by the method of Lowry et al. (18) using crystalline bovine albumin as
standard.

Procedure for Solubilization and Isolation of MEOS Activity—Mi-
crosomes were prepared as described above, except that the final pellet
of washed microsomes was covered with 0.25 M sucrose and stored
overnight under N₂ at −20°C. The microsomes were thawed before
further use, and the protein concentration was adjusted to 30 mg/ml
with 0.25 M sucrose. The solubilization of microsomes was achieved by
adding amounts to the buffer mixture described above. Most of the MEOS activity was
recovered, occasionally with traces of MEOS activity of low specific
activity. After KCl was added at a final concentration of 0.4 M to the
buffer mixture described above, most of the MEOS activity was
recovered. Eluates containing cytochrome P-450 and NADPH-cyto-
chrome c reductase activity were dialed overnight against 0.1 M
phosphate buffer (pH 7.0) and combined before further use (clarase
fraction).

The column was subsequently eluted (9) by adding KC1 in increas-
ing amounts to the buffer mixture described above. With the addition of KC1 at a final concentration of 0.1 M, some cytochrome P-450 was
recovered, occasionally with traces of MEOS activity of low specific
activity. After KC1 was added at a final concentration of 0.4 M to the
buffer mixture described above, most of the MEOS activity was
recovered. Eluates containing cytochrome P-450 and NADPH-cyto-
chrome c reductase activity were dialed overnight against 0.1 M
phosphate buffer (pH 7.0) and combined before the start of the incubations (MEOS fraction).

 rectangular Determinations—Alcohol dehydrogenase activity was
assayed after KC1 was added at a final concentration of 0.4 M to the
buffer mixture described above, most of the MEOS activity was
recovered. Eluates containing cytochrome P-450 and NADPH-cyto-
chrome c reductase activity were dialed overnight against 0.1 M
phosphate buffer (pH 7.0) and combined before the start of the incubations (MEOS fraction).

B: chemical Determinations—Alcohol dehydrogenase activity was
assayed after KC1 was added at a final concentration of 0.4 M to the
buffer mixture described above, most of the MEOS activity was
recovered. Eluates containing cytochrome P-450 and NADPH-cyto-
chrome c reductase activity were dialed overnight against 0.1 M
phosphate buffer (pH 7.0) and combined before the start of the incubations (MEOS fraction).

NADH-cytochrome b₅ reductase activity was estimated according to
the method of Omura and Sato (22). The activity of NADPH-cyto-
chrome c reductase was measured according to the method of Masters et al. (23).

NADPH-cytochrome b₅ reductase activity was determined according to
the method of Strittmatter (24) with potassium ferricyanide (0.25 mM)
as electron acceptor.

The activity of the microsomal alcohol-oxidizing system (MEOS)
determined with methanol, ethanol, propanol, and butanol as
substrates. Microsomes (4 to 6 mg of protein/flask) were preincubated
with the respective alcohol (methanol, 100 mM; ethanol, propanol, butanol, 50 mM) for 5 min at 37°C. The reaction was started by adding
the NADPH-generating system (0.4 mM NADPH, 8 mM sodium
isocitrate, and 0.34 unit/ml of isocitric dehydrogenase) and performed
in closed flasks. When a H2O2-generating system was employed, glucose
(10 mM) was preincubated with the microsomes and the alcohol,
and the reaction was started by addition of glucose oxidase
(0.7 to 1.0 mg/ml). The media contained 1.0 mM Na2EDTA, 5.0 mM
MgCl₂, and 0.1 M phosphate buffer (pH 7.4) in a final incubation
volume of 3.0 ml. The reactions were carried out for 0, 5, and 10 min
with duplicate determinations and were terminated by addition of 0.5
ml of 2% metaphosphoric acid (w/v). The samples were cooled and the samples were treated with trichloroacetic acid to a final concentration of 5%.

The absorbance at 224 nm was measured at 20°C. A standard curve was
prepared by adding known amounts of the respective aldehyde to the
incubation mixture at each time point. The spectra were identical with
those obtained for the aldehydes bound to the semicarbazide.

Product Identification and Quantitative Analysis by Absorption
Spectrophotometry and Gas-Liquid Chromatography—After the over-
night diffusion period, the semicarbazide solution of the center well
containing bound aldehydes was submitted to comparative absorption
spectrophotometry, using an Unicam SP 1800 Ultraviolet Spectrophotometer. Aliquots (0.3 ml) of the semicarbazide solution
were harvested and added to the sample cuvette which contained 2.7 ml
of distilled water. The reference cuvette contained 3.0 ml of distilled
water. In general, absorption spectra were recorded in a range of 190 to
270 nm for identification of the product formed upon oxidation of ethanol, propanol, or butanol by hepatic microsomes as well as for comparison with the respective commercially available aldehyde.

Qualitative identification and quantitative assessment of the alde-
hydes trapped by the semicarbazide were also performed by gas-liquid
chromatography, using a Perkin-Elmer F-40 gas-liquid chromato-
graph. In these experiments, the reactions were stopped by adding 0.5
ml of 70% perchloric acid (v/v). After an overnight diffusion period, 0.1
ml of the semicarbazide solution of the center well was added to 0.5 ml
of 35% perchloric acid (v/v) contained in the 25ml glass flask to be
used for the chromatograph. The resulting supernatant was used for the formaldehyde
identification. The incubation medium was centrifuged at 3000 x g
for 10 min. Erlenmeyer flasks. After stopping the reaction with trichloroacetic acid, the absorption at 224 nm was observed, when the semicarbazide solution of the center well was tested after an overnight diffusion period. The absorption spectra were identical with those obtained
for the aldehydes bound to the semicarbazide. The spectrum of the semicarbazide
solution did not change when the semicarbazide solution was added to the
reference cuvette. The concentration of formaldehyde in the incubation medium was
estimated according to the method of Nash (25). The reactions were carried out in 50-ml
Erlenmeyer flasks. After stopping the reaction with trichloroacetic acid, the
incubation medium was centrifuged at 3000 x g for 10 min. Aliquots of the resulting supernatant were used for the formaldehyde
determination. The incubations with ethanol, propanol, and butanol
as substrates were carried out in 50-ml Erlenmeyer flasks with center
wells containing 0.6 ml of 15 mM semicarbazide hydrochloride in 0.1
M phosphate buffer (pH 7.4) as described by Lieber and DeCarli (3). The
aldehydes bound to the semicarbazide were determined at 224 nm after
an overnight diffusion period at room temperature. With each incuba-
tion set flasks were incorporated to which known amounts of aldehydes
were added in the incubation medium to be used as standards.

RESULTS

Statistical Analysis—Each individual result was compared with the value of its corresponding control, and the means (±S.E.) and
individual differences were calculated. Their significances were as-
Analyzed by Student's t test.

Assay for Activity of Microsomal Alcohol-oxidizing System
(MEOS)—Hepatic microsomes were found to actively oxidize
a variety of primary alcohols (Table I). Indeed after incubation
of ethanol, propanol, or butanol with microsomes and a
NADPH-generating system, an increase in absorbance at 224
nm was observed, when the semicarbazide solution of the
center well was tested after an overnight diffusion period.
These absorption spectra were identical with those obtained
The activity of MEOS with all four alcohols as substrates was found to be proportional to the amount of protein employed throughout this study. In a set of experiments, the activity of MEOS increased linearly with time for at least 10 min. The apparent $K_m$ values determined for various alcohols decreased with increasing chain length of the alcohols used as substrates: methanol (22.2 mM), ethanol (9.6 mM), propanol (5.5 mM), and butanol (4.9 mM). The $V_{max}$ values for methanol and ethanol as substrates were approximately twice that for propanol and butanol (Fig. 1).

Studies with Inhibitors of MEOS Activity—Experiments were carried out with the NADPH-generating system and with various alcohols in different concentrations ranging from 5 to 20 mM. Double-reciprocal plots obtained according to the method of Lineweaver-Burk were linear in the concentration range tested in this study (Fig. 1). The apparent $K_m$ values for methanol and ethanol as substrates were approximately twice that for propanol and butanol (Fig. 1).

Studies with Inhibitors of MEOS Activity—Microsomes were incubated under an atmosphere containing CO (40%), O$_2$ (4%), and N$_2$ (56%). CO strikingly inhibited MEOS activity by 40 to 50% compared to control incubations carried out under O$_2$ (4%) and N$_2$ (96%) (Table II). The inhibitory effect of CO was somewhat greater with propanol and butanol as substrates than with methanol and ethanol. Under experimental conditions in which air was replaced by N$_2$ (100%) flushed for 5 min prior to the start of the reaction, MEOS activity was almost completely lost.

Sodium azide (0.1 mM), a catalase inhibitor (26), decreased MEOS activity only with methanol and ethanol as substrates, but no inhibitory effect was observed with propanol and butanol under these experimental conditions (Table II). Subsequently, a titration curve was performed with sodium azide in concentrations up to 1.0 mM (Fig. 2). Increasing the concentrations of sodium azide above 0.1 mM failed to further diminish.
Microsomes (6.0 mg of protein/flask) were preincubated with the respective alcohol (in final concentrations as indicated) at 37°C for 5 min. The reaction was started by adding the NADPH-generating system (0.4 mM NADP+, 8 mM sodium isocitrate, and 0.34 unit/ml of isocitric dehydrogenase). The incubations were carried out at 37°C in an incubation medium (final volume 3.0 ml) containing 0.1 mM phosphate buffer (pH 7.4), 1 mM EDTA, and 5 mM MgCl₂.

**TABLE II**

Effect of CO on MEOS activity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>CO</th>
<th>Inhibition</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>7.2 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>40.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.7 ± 0.6</td>
<td>4.4 ± 0.2</td>
<td>40.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Propanol</td>
<td>4.2 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>52.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Butanol</td>
<td>3.0 ± 0.4</td>
<td>1.6 ± 0.1</td>
<td>46.7</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

MEOS activity almost completely (Table I), possibly by converting cytochrome P-450 to its inactive form P-420 (29). Similar results were obtained with p-chloromercuribenzoate (Table I) which reacts preferentially with sulfhydryl groups (29). Finally, addition of urea, a membrane-disrupting agent, had a slight inhibitory effect on MEOS activity (Table I).

**Role of H₂O₂ Generation in Microsomal Alcohol Oxidation**

-Microsomes were incubated with a H₂O₂-generating system to assess the peroxidatic activity of catalase for various alcohols. Methanol and ethanol were oxidized at similar rates whether the alcohol was incubated with the NADPH-generating system or the H₂O₂-producing one (Table III). With propanol and butanol as substrates, substantial metabolic rates were observed only with the NADPH-generating system, whereas negligible or no oxidation was demonstrated in the presence of a H₂O₂-generating system (Table III). These results confirm that both propanol and butanol are extremely poor or virtually not substrates for catalase-H₂O₂.

Methanol and ethanol are metabolized not only by generated NADPH, but also by a H₂O₂-producing system (Table III), and the kinetic characteristics of these reactions were compared. The incubations were carried out with alcohol concentrations ranging from 10 to 50 mM. The double-reciprocal plots obtained according to Lineweaver-Burk were linear in the range tested.
and revealed an apparent $K_m$ value for ethanol of 9 mM with a NADPH-generating system and of 40 mM with a H$_2$O$_2$ generating system (Fig. 3). Thus, the kinetic characteristics of the NADPH-dependent ethanol oxidation are strikingly different from those of the H$_2$O$_2$-mediated process (Fig. 3). When methanol was used as substrate, the apparent $K_m$ values for methanol were 25 mM with NADPH and 37 mM with H$_2$O$_2$.

**Isolation of MEOS Activity by DEAE-cellulose Column Chromatography**—Following the solubilization of hepatic microsomes and subsequent ion-exchange column chromatography on DEAE cellulose, the void volume contained catalase as measured by its catalatic property (catalase fraction). Eluates recovered with 0.4 mM KCl contained microsomal components cytochrome P-450, NADPH-cytochrome c reductase, cytochrome $b_5$ and NADH-cytochrome $b_5$ reductase but were completely devoid of alcohol dehydrogenase as well as catalatic activity (MEOS fraction). In the latter fraction, a striking NADPH-dependent oxidation was observed with methanol, ethanol, propanol, and butanol as substrates (Table IV). However, none of these alcohols was oxidized to its respective aldehyde with the H$_2$O$_2$-generating system (Table IV), a finding which differs from the experiments carried out in intact hepatic microsomes (Table III). These results therefore indicate that the MEOS fraction was completely free of peroxidatic activity of catalase. The employed H$_2$O$_2$-generating system was active as shown by its incubation with the catalase fraction: a striking oxidation of methanol and ethanol was observed in the presence of the H$_2$O$_2$-generating system (Table IV). These results also show that catalase retained its peroxidatic property following solubilization of microsomes and column chromatography; the lack of detectable peroxidatic activity of catalase in the isolated MEOS fraction clearly indicated the catalase-independent nature of MEOS in this experiment. Furthermore, no substantial oxidation of propanol and butanol was demonstrated in the catalase fraction with the H$_2$O$_2$ generating system (Table IV), indicating that these two alcohols are not substrates for catalase-H$_2$O$_2$. As expected, none of the alcohols was metabolized in the presence of the NADPH-generating system by the catalase fraction, in which no microsomal components could be detected.

**DISCUSSION**

The capacity of rat liver microsomes to oxidize methanol, ethanol, propanol, and butanol to their respective aldehydes is described. This microsomal alcohol-oxidizing system (MEOS) is NADPH-dependent (Table I) and requires molecular oxygen; it therefore shares some characteristics with other microsomal drug oxidations (31). The partial inhibition of MEOS activity by CO (Table II) may implicate the involvement of cytochrome P-450. It is noteworthy that the inhibition of MEOS activity by CO is somewhat lower than that reported for some other microsomal drug-detoxifying enzymes (32). The reason for this is unknown. The difference may be explained by the unusual low affinity for CO of Form I of cytochrome P-450 (33), the species which has been considered to play a role in the microsomal ethanol-oxidizing system (MEOS) (34). In the present study, MEOS activity was virtually abolished in the presence of sodium cholate and sodium deoxycholate (Table I), compounds which are known to convert cytochrome P-450 to its inactive form P-420 (29). Furthermore, compounds which bind to cytochrome P-450 such as imidazole inhibit MEOS activity (Table I). An interaction of alcohols with cytochrome P-450 is also suggested by the observation that addition of methanol, ethanol, propanol, and butanol to a microsomal suspension results in the formation of a modified type II binding spectrum (35, 36). Interestingly, the spectral change increases with increasing chain length of the alcohol (35) findings which are in keeping with the enhanced lipophilicity of higher aliphatic alcohols. Similarly, the inhibition of a cytochrome P-450-dependent reaction such as ethylmorphine demethylase was much more pronounced with butanol than with ethanol (37). Nevertheless, the rates of the NADPH-dependent alcohol oxidation decreased with increasing chain length of the alcohol (Fig. 1), findings which might support the contention that alcohols and ethylmorphine are not metabolized at a common site of cytochrome P-450 or even that cytochrome P-450 may not be involved in the microsomal alcohol oxidation. However, alcohols not only bind to, but they

**Table IV**

**Effect of NADPH and H$_2$O$_2$ generation on alcohol oxidation catalyzed by column fractions**

<table>
<thead>
<tr>
<th>Alcohols</th>
<th>Catalase fraction</th>
<th>MEOS fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H$_2$O$_2$</td>
<td>NADPH</td>
</tr>
<tr>
<td>Methanol</td>
<td>16.8</td>
<td>11.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.6</td>
<td>26.6</td>
</tr>
<tr>
<td>Propanol</td>
<td>0.3</td>
<td>16.6</td>
</tr>
<tr>
<td>Butanol</td>
<td>0</td>
<td>10.6</td>
</tr>
</tbody>
</table>
also influence the lipid environment of cytochrome P-450, and changes in the hydrophobic milieu may further contribute to alteration in the strength of the ligand association with the heme iron (37). Thus, it appears that factors other than binding to cytochrome P-450 may determine the over-all rate of microsomal alcohol oxidation.

Apart from cytochrome P-450, NADPH cytochrome c reductase has been considered as an essential component of the NADPH-dependent microsomal drug hydroxylation system (19). Previously it was shown that, in the absence of cytochrome P-450, NADPH-cytochrome c reductase failed to promote the oxidation of ethanol to acetaldehyde (7-9). However, as in the case of microsomal drug oxidation, addition of NADPH-cytochrome c reductase to cytochrome P-450 resulted in a striking increase of the activity of the microsomal ethanol-oxidizing system (MEOS), indicating that the reductase plays a role in microsomal alcohol oxidation (8). Thus, the NADPH-dependent microsomal electron transport chain consisting of both NADPH-cytochrome c reductase as well as cytochrome P-450 may participate, at least in part, in the activity of the microsomal alcohol-oxidizing system. This is supported by the finding that MEOS is most active with NADPH or a NADPH-generating system (Table I). Furthermore, no synergism of NADPH and NADHD could be established for MEOS activity (Table I), indicating that the reaction proceeds at a maximal rate when electrons were donated solely by NADPH. This is in agreement with similar properties described for the microsomal metabolism of type II binding substrates, whereas type I binding compounds are metabolized at higher rates when both NADPH and NADHD are present (38, 39).

Hepatic microsomes metabolize alcohols also in the presence of NADH, although at a slower rate compared to the NADPH-dependent reaction (Table I). Since NADH is the main electron donor for the microsomal electron transport chain involving NADH cytochrome b5 reductase and cytochrome b5 (38, 40), the results suggest that the latter two microsomal components are involved in the NADPH-dependent MEOS activity. Moreover, NADPH can also provide electrons to cytochrome b5 reductase and cytochrome b5 (40). Indeed, it has been shown that NADPH promotes the oxidation of ethanol in the presence of isolated cytochrome b5 reductase and cytochrome b5 (41); thus this pathway may contribute to the over-all process of microsomal alcohol oxidation. Since various mechanisms involving both of the microsomal electron transport chains can be implicated in the NADPH-dependent MEOS activity, further studies are necessary to establish quantitatively the role of each of the proposed pathways.

The exact molecular basis of MEOS activity is still unknown. The production of hydroxyl radicals has been described in hepatic microsomes upon oxidation of NADPH (42). Since ethanol is a potent scavenger for hydroxyl radicals (43, 44), it is conceivable that this form of activated oxygen promotes the NADPH-dependent oxidation of alcohols in microsomes. Evidence for the latter mechanism is provided by the fact that formate, another scavenger for hydroxyl radicals (27), inhibits the NADPH-dependent alcohol oxidation catalyzed by whole hepatic microsomes (Table I) and by isolated microsomal components (9). Similarly, hydroxyl radicals have been considered to play some role in the ethanol oxidation promoted by cytochrome b5 reductase and cytochrome b5 (41).

The present study clearly differentiates the microsomal alcohol-oxidizing system from catalase-H2O2 by a variety of characteristics. This is of particular importance since microsomes are contaminated by catalase which exhibits properties identical with those of peroxysomal catalase. As it is the case for the peroxysomal variety (45), catalase of the microsomal fraction is characterized by its capacity to decompose H2O2 catalytically (3) and to peroxidize lower aliphatic alcohols in the presence of an active H2O2-generating system (Table III). Furthermore, the catalatic as well as peroxidatic activity of catalase is strongly inhibited by sodium azide in both peroxysomes (45) and in microsomes (3). These findings are consistent with the contentions of others (46) that catalase is not a constituent of the microsomal membranes itself, but rather represents a contamination of peroxysomal catalase.

In agreement with some (4-9) but not all previous reports (13, 14), the data presented in this study definitively rule out an obligatory role of catalase-H2O2 in the microsomal alcohol-oxidizing system. Indeed, the NADPH-dependent microsomal system oxidizes lower as well as higher aliphatic alcohols (Tables I to IV) whereas catalase contaminating the microsomal fraction metabolizes only lower aliphatic alcohols with a H2O2-generating system (Table III), in keeping with similar properties described for catalase of the peroxysomal fraction (15). These findings differentiate the NADPH-dependent microsomal alcohol-oxidizing system from the catalase-H2O2-mediated alcohol oxidation by substrate specificity and rule out an obligatory or even facultative role of catalase-H2O2 in the microsomal oxidation of higher aliphatic alcohols. This concept is supported by the failure of the catalase inhibitor sodium azide to decrease the NADPH-dependent microsomal oxidation of propanol and butanol (Table I, Fig. 2). Finally, the NADPH-dependent propanol and butanol oxidation could be demonstrated in the isolated MEOS fraction in the absence of catalase (Table IV). The data presented are at variance with a previous report (47) in which no aldehyde formation from higher aliphatic alcohols such as propanol and butanol was detected in porcine microsomes. This discrepancy is most probably due to differences in the method used to assay for aldehydes. Since catalase does not react peroxidatically with higher aliphatic alcohols (12), the failure of detectable oxidation of propanol and butanol by hepatic microsomes (47) was previously considered by others (14) as strong evidence for an obligatory role of catalase-H2O2 in the microsomal alcohol oxidation, an interpretation which requires reconsideration in view of the microsomal oxidation of propanol and butanol observed in the present study.

In contrast to higher aliphatic alcohols, methanol and ethanol are substrates for catalase-H2O2 (Table III), suggesting some facultative role of catalase in the NADPH-dependent oxidation of methanol and ethanol in intact liver microsomes. Indeed, the catalase inhibitor sodium azide partially inhibits the microsomal oxidation of lower aliphatic alcohols as shown in the present study (Table I, Fig. 2) as well as in previous reports (3, 13, 17, 48, 49); the degree of inhibition by 0.1 mM azide varied in these studies from 10 to 50% depending on the assay conditions, the amount of catalase contamination of the microsomal fraction and on the rates of microsomal H2O2 generation. However, in view of the extremely low rate of microsomal H2O2 generation (50) and the only partial inhibition of the microsomal methanol and ethanol oxidation by sodium azide (Table I, Fig. 2) it appears that the role of catalase for microsomal alcohol oxidation is of limited importance. Furthermore, the striking difference of the apparent Km values for ethanol between the NADPH-dependent and the
catalase-\(\text{H}_2\text{O}_2\)-mediated ethanol oxidation observed in the present study (Fig. 3) contrasts with the finding of Thurman et al. (14) who reported identical \(K_m\) values for microsomal ethanol oxidation whether a NADPH-generating system was used or a \(\text{H}_2\text{O}_2\)-producing one. Finally, an obligatory involvement of catalase-\(\text{H}_2\text{O}_2\) in the NADPH-dependent microsomal oxidation of lower aliphatic alcohols is definitively excluded. Indeed, upon isolation of the microsomal alcohol-oxidizing system by DEAE-cellulose column chromatography no alcohol oxidation could be observed in the MEOS fraction with an active \(\text{H}_2\text{O}_2\)-generating system, whereas the rates of the NADPH-dependent alcohol oxidation persisted (Table IV). These results therefore show that the isolated MEOS fraction was indeed free of peroxidatic activity of catalase and nevertheless retained its capacity to metabolize methanol, ethanol, propanol, and butanol with NADPH. Conversely, catalase which contaminates intact microsomes was eluted during the column chromatographic procedure in the void volume and retained its capacity of oxidizing lower aliphatic alcohols with a \(\text{H}_2\text{O}_2\)-generating system (Table IV). Therefore, contrasting with the catalase-\(\text{H}_2\text{O}_2\)-mediated alcohol oxidation, MEOS was not active with generated \(\text{H}_2\text{O}_2\) (Table IV) which further differentiates both enzyme systems.

In the present study, the isolation of MEOS activity was carried out with livers of rats fed Purina Laboratory Chow (Table IV). Similar results were obtained in a recent collaborative study using rats fed an ethanol-containing liquid diet for 5 weeks. In the latter study neither catalatic nor peroxidatic activity was detected in the isolated microsomal fraction following DEAE-cellulose column chromatography when tested under a variety of experimental conditions. Furthermore, whereas generated \(\text{H}_2\text{O}_2\) was completely ineffective, NADPH promoted the ethanol oxidation in the isolated MEOS fraction, resulting in striking rates of acetaldehyde production. Therefore, the claim of Thurman et al. (14) that the microsomal alcohol-oxidizing system is due exclusively to catalase-\(\text{H}_2\text{O}_2\) in a reaction in which the \(\text{H}_2\text{O}_2\) generation is the rate-limiting step could not be substantiated.

The microsomal alcohol oxidizing system was also differentiated from cytosolic alcohol dehydrogenase by a variety of characteristics. MEOS activity requires NADPH as cofactor (Table I) whereas cytosolic alcohol dehydrogenase is predominantly active with NAD\(^+\). Furthermore, it was shown that MEOS activity is insensitive to pyrazole (Table I), an inhibitor for cytosolic alcohol dehydrogenase (30). Differentiation of MEOS from cytosolic alcohol dehydrogenase was also shown by the fact that ethanol is oxidized by MEOS at a higher rate than propanol or butanol (ethanol > propanol > butanol) both in intact microsomes (Fig. 1) and in the isolated MEOS fraction (Table IV), whereas the reverse is observed for cytosolic alcohol dehydrogenase of rat liver (51). Furthermore, the kinetic properties of MEOS differ substantially from those of cytosolic alcohol dehydrogenase. The \(K_m\) values of MEOS (Fig. 1) are 9.6 \(\text{mM}\) for ethanol and 4.9 \(\text{mM}\) for butanol, whereas the corresponding values reported for cytosolic alcohol dehydrogenase are 0.5 to 2 \(\text{mM}\) for ethanol and 9 \(\text{mM}\) for butanol (52, 53). Although MEOS exhibits characteristics which differ from those of cytosolic alcohol dehydrogenase, one might argue that microsomes contain a membrane-bound alcohol dehydrogenase with different properties than those of the cytosolic form. Indeed, unwashed microsomes contain trace amounts of alcohol dehydrogenase activity, but the activity of the microsomal alcohol-oxidizing system remained unaffected when alcohol dehydrogenase activity was removed completely by DEAE cellulose column chromatography of solubilized microsomes (17). Furthermore, in the present study, washed rather than unwashed microsomes were used, since the former contain no alcohol dehydrogenase activity (4). The alcohol dehydrogenase-independent nature of MEOS was verified further in the present study by the failure to detect any alcohol dehydrogenase activity in column fractions exhibiting striking MEOS activity (Table IV).

The demonstration that hepatic microsomes are capable of oxidizing various alcohols to their respective aldehydes at a physiological pH raises the question of the role of this additional system in alcohol metabolism in vivo. The apparent \(K_m\) values of the microsomal alcohol-oxidizing system in vitro (Fig. 1) for ethanol (9.6 \(\text{mM}\)), propanol (5.5 \(\text{mM}\)), and butanol (4.9 \(\text{mM}\)) indicate that, if applicable to a situation in vivo this microsomal system could be near its maximum activity with blood alcohol concentrations found after alcohol administration. It is therefore conceivable that the microsomal alcohol-oxidizing system accounts, at least in part, for the alcohol dehydrogenase-independent pathway of alcohol metabolism in vivo (64, 55) as well as in vitro in perfused liver (56), liver slices (3, 57), and isolated parenchymal liver cells (58, 59).

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