# ALCOHOL MIXED FUNCTION OXIDASE ACTIVITY OF MAMMALIAN LIVER MICROSOMES*

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Previously reported mammalian liver alcohol dehydrogenases have been soluble enzymes catalyzing the reduction of $NAD^+$ with concurrent production of aldehyde (Sund and Theorell, 1963; Von Wartburg et al., 1964). In extending our studies on microsomal oxidative N-dealkylation (Pettit et al., 1964), we have observed that mammalian liver microsomes can catalyze the NADPH and oxygen dependent oxidation of methanol and ethanol to the corresponding aldehydes. Higher primary and secondary alcohols apparently are not oxidized by this system. This particulate alcohol oxidase may furnish an alternate pathway for the metabolism of the lower alcohols, especially in tissues such as horse liver, where the soluble alcohol dehydrogenase does not catalyze the oxidation of methanol. In addition, the simplicity of this substrate-product pair may make it valuable in the study of the mechanism of the microsomal mixed-function oxygenases (Schuster, 1964; Mason, 1965).

The microsomes and other cell fractions were isolated by differential centrifugation from pork, rabbit, and rat liver homogenates by a modification of the method of Hogeboom et al., (1948). The enzyme assays were carried out in open 10 ml Erlenmeyer flasks at 38° in a Dubnoff metabolic shaker. The compositions of the assay media are given in the Tables. After preincubation for four minutes, the reaction was started by adding either the microsomes or substrate. Aliquots of the reaction mixture were taken at 5 minute intervals and deproteinized with perchloric acid. The deproteinized extracts were assayed

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for formaldehyde, (Nash, 1953), aldehyde (Albrecht et al., 1963), or ketone (Bernt sson, 1956).

That the microsomal methanol oxidizing system requires both NADPH and oxygen is evident from the data of Table 1. Like the oxidative N-dealkylation sy-

TABLE 1
Requirement of Methanol Oxidase of Pork Liver Microsomes

| Composition of Reaction Medium | mμmoles formaldehyde formed per mg protein per minute |
|---|---|
| Complete [1] | 2.4 |
| Minus NADPH | 0.1 |
| Minus methanol | 0.05 |
| Minus $O_2$ ($N_2$ atmosphere) | 0.0 |
| Minus microsomes plus boiled microsomes | 0.0 |
| Minus NADPH plus NADH | 0.8 |
| Minus NADPH plus $NADP^+$ | 0.3 |
| Minus NADPH plus $NAD^+$ | 0.4 |
| Minus NADPH plus $H_2O_2$ | 0.3 |
| Complete plus $H_2O_2$ | 2.6 |

[1] 50 μmoles methanol, 0.5 μmole NADPH, 2.5 μmoles semicarbazide, 100 μmoles phosphate buffer, pH 7.0, and ca. 5 mg microsomal protein, all per ml. Pyridine nucleotides (0.5 μmoles per ml) and hydrogen peroxide (5 μmoles per ml) were added where indicated.

stems, NADH at the same concentration is far less effective in promoting methanol oxidation than is NADPH. $NAD^+$ and $NADP^+$ are only slightly effective at the concentrations tested, indicating that the system differs from classical alcohol dehydrogenases. In addition to methanol, the oxidase present in rat, rabbit, and pork liver microsomes will also catalyze the oxidation of ethanol at about 50 per cent of the methanol rate. Oxidation of n-propanol, iso-propanol, n-butanol, n-amyl alcohol, or s-butanol could not be detected and, with the exception of ethanol, the oxidation of methanol was not inhibited when both methanol and a higher alcohol were present at $5X10^{-2}$ M.

The NADPH and oxygen dependent alcohol oxidase activity appears to be concentrated in the microsome fraction of liver homogenates (Table 2). The

TABLE 2
Methanol Oxidase Activity of Subcellular Fractions of Liver Homogenates

| Species | Specific Activity[1] mitochondria | microsomes | supernatant |
|---|---|---|---|
| Pig | 0.74 | 3.8 | 0.82 |
| Rabbit | 0.07 | 0.77 | 0.07 |
| Rat | 0.49 | 2.8 | 0.39 |

[1] Expressed as mμmoles formaldehyde formed per mg protein per minute; in the presence of 0.50 μmole NADP, 5 μmoles each $MgCl_2$ and sodium isocitrate, 2.5 μmoles semicarbazide, isocitric dehydrogenase, 50 μmoles methanol, 100 μmoles phosphate buffer, and ca. 5 mg protein, all per ml.

marginal activity observed in supernatant and mitochondrial fractions has not been extensively studied. The methanol oxidation observed in these fractions could be due to microsomal contamination.

The microsomal methanol oxidase could be formulated as the production of hydrogen peroxide via NADPH-oxidase followed by peroxydatic attack on the alcohol (Omura and Sato, 1964). However, this mechanism is unlikely in view of the pyridine nucleotide specificity (Table 1) of the microsomal oxidase. The rate of hydrogen peroxide generation by the microsomal NADH-oxidase is as great, or greater, than that of the analogous NADPH-oxidase (Modirzadeh and Kamin, 1965). Furthermore, hydrogen peroxide per se does not appear to be involved in the reaction, since, as shown in Table 1, hydrogen peroxide added in 10-fold excess to the NADPH is only 15 per cent as effective as NADPH alone, and the NADPH and hydrogen peroxide dependent methanol oxidation rates are additive.

The effects of some oxidase inhibitors on the pork liver microsomal methanol oxidase are listed in Table 3. The sulfhydryl binding agent p-chloromercuribenzoate at $10^{-3}$M concentration produced nearly complete inhibition of

activity. Carbon monoxide, at a gas-phase concentration ratio of 4:1 to oxygen, caused little if any diminuation of activity, nor did the diethylaminoethanol-ester of diphenylpropylacetic acid (SKF525-A) at $5X10^{-4}$ M. These effects parallel those found for the oxidative N-demethylation of N,N-dimethylaniline in the same systems.[+] In contrast to the oxidative N-dealkylating system, the alcohol oxidase is inhibited by sodium azide and sodium cyanide. From these data, it would appear that the alcohol oxidase is a separate system from the microsomal oxidative N-dealkylase.

TABLE 3
Effects of Inhibitors on Pork Liver Microsomal Methanol Oxidase Activity

| | Concentration | % Inhibition[1] of Methanol oxidation |
|---|---|---|
| p-chloromercuribenzoate | $10^{-3}$ M | 92 |
| 4,4,4,trifluoro-1-(2-thienyl)-1,3-butanedione | $10^{-3}$ M | 21 |
| carbon monoxide | $[CO]/[O_2]$ = 4 in the gas phase | 0 |
| SKF525-A | $5X10^{-4}$ M | 0 |
| sodium azide | $10^{-3}$ M | 76 |
| sodium cyanide | $10^{-3}$ M | 68 |

[1] Reaction medium was the same as the complete system of Table 1, supplemented with inhibitors at the concentration indicated.

The data presented in this paper support the hypothesis that mammalian liver microsomes are capable of catalyzing the oxidation of lower alcohols by a pathway radically different from the well-known soluble dehydrogenase systems. The quantitative significance and mechanism of this oxidation remain to be determined.

[+] Ziegler and Orme-Johnson, unpublished observations.

## REFERENCES

Albrecht, A. M., Sher, W. S., and Vogel, H. H., Anal. Chem. 34, 398 (1962).
Berntsson, S., Anal. Chem. 28, 1337 (1956).
Hogeboom, G. H., Schneider, W. C., and Palade, G. F., J. Biol. Chem. 172, 619 (1948).
Mason, H. S., Ann. Rev. Biochem. 34, 595 (1965).
Modirzadeh, J., and Kamin, H., Biochem. et Biophys Acta 99, 205 (1965).
Nash, T., Biochem. J. 55, 416 (1953).
Omura, R., and Sato, R., J. Biol. Chem. 239, 2370 (1964).
Pettit, F. H., Orme-Johnson, W. H., and Ziegler, D. M., Biochem. Biophys. Res. Comm. 16, 444 (1964).
Schuster, L., Ann. Rev. Biochem. 33, 571 (1964).
Sund, H., and Theorell, H., The Enzymes, Vol. 7, Boyer et al., eds., Academic Press, New York, 1963, p. 25.
Von Wartburg, J. P., Bethune, J. L., and Vallee, B. L., Biochem. 3, 1775 (1964).