# THE CATALYTIC DEMETHYLATION OF N,N-DIMETHYLANILINE-N-OXIDE BY LIVER MICROSOMES*

Flora H. Pettit and Daniel M. Ziegler+

Clayton Foundation Biochemical Institute and the Department of Chemistry
The University of Texas, Austin, Texas

Received August 23, 1963

The enzyme system present in mammalian liver tissue catalyzing the oxidative demethylation of lipid soluble N-methyl compounds was first described by Mueller and Miller (1953). Subsequent studies by LaDu, *et al*. (1955) demonstrated that the enzyme system was localized in the microsomal fraction of liver homogenates. The isolated microsomes when supplemented with NADPH and oxygen catalyzed the oxidative dealkylation of a variety of lipid soluble N-alkyl compounds to the aldehyde and corresponding amine (Gillette *et al*. 1957). The enzyme system appears to be of the type classified by Mason (1957) as a mixed function oxidase. It has been suggested (*cf*. Brodie 1958) that in the first part of the reaction the N-methyl compound is oxidized to the alkyl N-oxide and the intermediate N-oxide is then degraded as indicated by the last two steps in the following sequence of reactions:

$$\text{i)}\quad R\text{-}N(CH_3)_2 \xrightarrow[O_2]{NADPH} R\text{-}\overset{O}{\overset{\uparrow}{N}}(CH_3)_2$$

$$\text{ii)}\quad R\text{-}\overset{O}{\overset{\uparrow}{N}}(CH_3)_2 \longrightarrow R\text{-}N\begin{matrix}\diagup CH_2OH\\ \diagdown CH_3\end{matrix}$$

$$\text{iii)}\quad R\text{-}N\begin{matrix}\diagup CH_2OH\\ \diagdown CH_3\end{matrix} \longrightarrow R\text{-}\overset{H}{\overset{|}{N}}CH_3 + H_2C{=}O$$

---

*Supported in part by a grant from National Institutes of Health, GM-09044-02.

+Work carried out during tenure as an Established Investigator, American Heart Association, Inc.

While there was some evidence to support this reaction mechanism (Fish et al. 1955,1956), an earlier report indicated that the rate of N,N-dimethylaniline-N-oxide demethylation was too slow for it to be an intermediate in the oxidative demethylation of N,N-dimethylaniline by liver microsomes (cf. Brodie 1958). The data presented in this report demonstrate that rat and pig liver microsomes catalyze the rapid demethylation of N,N-dimethylaniline-N-oxide to methylaniline and formaldehyde in the absence of both NADPH and oxygen. The maximum rate of this reaction is several fold greater than the overall oxidative demethylation of dimethylaniline by the liver microsomes.

The microsome fractions were isolated by differential centrifugation from liver homogenates prepared in 0.25 M sucrose according to the method of Hogeboom et al. (1948).

The oxidative demethylation assays were carried out in open 10 ml erlenmeyer flasks at 38° in a Dubnoff metabolic shaker. The complete reaction medium contained per ml: potassium phosphate, pH 7.5, 200 μmoles; magnesium chloride, 5 μmoles; isocitrate, 5 μmoles; $NAD^+$, 5 μmoles; $NADP^+$, 0.1 μmole; semicarbazide, 1 μmole; and sufficient isocitric dehydrogenase to reduce 0.5 μmole of $NADP^+$ per minute per ml. The concentration of N,N-dimethylaniline, N,N-dimethylaniline-N-oxide and of microsomal protein are given in the Table and Figure. After preincubating with shaking for 4 minutes the reaction was started by adding either the microsomes or the substrate. Aliquots of the reaction mixture were withdrawn at intervals and pipetted into tubes containing sufficient 6.0 M trichloroacetic acid to give a final concentration of 0.6 M. The deproteinized supernatant solutions were assayed for formaldehyde by the method of Nash (1953). The rates of formaldehyde formation reported in this paper are corrected for the small amount of formaldehyde produced in the absence of substrate. N-methylaniline was estimated by vapor phase chromatography using a column packed with 5 per cent polyethylene glycol succinate on chromosorb P at 182°.

Commercial N,N-dimethylaniline was distilled under vacuum. Analysis by vapor phase chromatography indicated that it was free from contaminants other than traces of N-methylaniline. The N,N-dimethylaniline-N-oxide was synthesized by Dr. Rowland Pettit of our Department. The melting point of the recrystallized N-oxide was 150° [reported, 152° (Belov and Savich 1947)].

The data summarized in Table I demonstrate that pig liver microsomes catalyze the demethylation of N,N-dimethylaniline-N-oxide at a rate greater than the oxidative demethylation of the corresponding dimethyl amine. Vapor phase chromatography of ether extracts (at pH 9.0) of the reaction medium indicate that N-methylaniline is produced at approximately the same rate as formaldehyde during the catalytic demethylation of the N-oxide. Recent experiments indicate that the microsomal component catalyzing the demethylation of the N-oxide is heat labile, non-dializable and appears to be an enzyme. The rate of formaldehyde production is a linear function of microsomal protein concentration over a relatively wide range (0.2 to 4.0 mg per ml) and the reaction is also linear with time for at least the first ten minutes.

A graphical determination of the Michaelis constant for N,N-dimethylaniline in rat liver microsomes gave a $K_m$ of $1.42 \times 10^{-3}$ $\underline{M}$. The Michaelis constant for the N-oxide was determined in both rat and pig liver microsomes from the Lineweaver-Burke plot shown in Figure I. The high $K_m$ value ($139 \times 10^{-3}$ $\underline{M}$) observed with both rat and pig liver microsomes may be due to the very limited lipid solubility of the polar N-oxide (cf. Brodie 1958).

The data presented in this report demonstrates that the rate of N,N-dimethylaniline-N-oxide demethylation by liver microsomes is sufficiently high for the N-oxide to be considered as an intermediate in the oxidative demethylation of N,N-dimethylaniline.

Table I

Demethylation Activity of Pig Liver Microsomes

| Additions | | Substrates: N,N-dimethyl-aniline | Substrates: N,N-dimethyl-aniline-N-oxide |
|---|---|---|---|
| | | mμmoles formaldehyde per min per mg | |
| Complete system | | 5.4 | 15 |
| " " | minus $NADP^+$ and NADPH generating system | 0 | 14 |
| " " | minus NADPH generating system and oxygen | 0 | 17 |
| " " | minus NADPH generating system, $NAD^+$ and $MgCl_2$ | 0 | 17 |
| " " | minus microsomes | 0 | 0 |
| " " | minus microsomes plus boiled microsomes | 0 | 0 |

Concentration of substrates, 3 μmoles per ml; concentration of microsomal protein, 2.6-3.3 mg per ml.

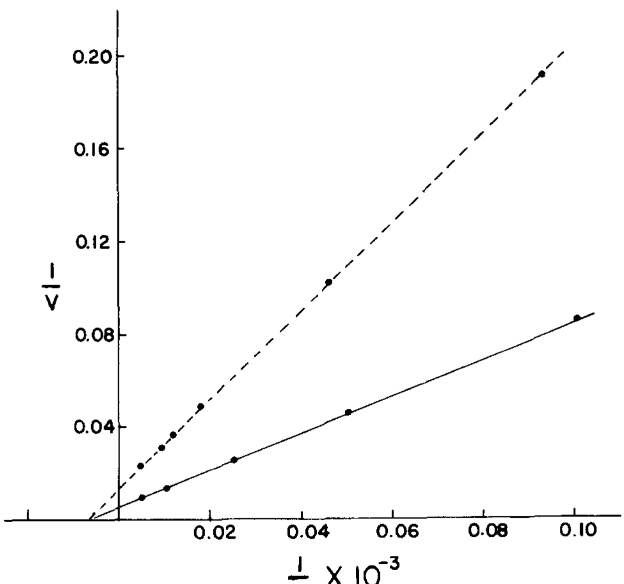


Figure I. Lineweaver-Burke plot of the effect of substrate concentration on the rate of demethylation of N,N-dimethylaniline-N-oxide.

[S] = Molarity

V = mμmoles formaldehyde produced per min. per mg microsomal protein

—— = Pig liver microsomes $K_m = 139 \times 10^{-3}$M $V_{max}$ = 175.4 mμmoles per min per mg

--- = Rat liver microsomes $K_m = 139 \times 10^{-3}$M $V_{max}$ = 71.4 mμmoles per min per mg

## References


1. Belov, V. N., and Savich, K. K., J. Gen. Chem. (U.S.S.R.), 17, 257 (1947).

2. Brodie, B. B., Ann. Rev. Biochem., 57, 427 (1958).

3. Fish, M. S., Johnson, N. M., Lawrence, E. R., Horning, E. C., Biochim. et Biophys. acta, 18, 564 (1955).

4. Fish, M. S., Sweeley, C. C., Johnson, N. M., Lawrence, E. P., and Horning, E. C., Biochim. et Biophys. acta, 21, 196 (1956).

5. Gillette, J. R., Brodie, B. B., and LaDu, B. N., J. Pharmacol. Exptl. Therap., 119, 532 (1957).

6. Hogeboom, G. H., Schneider, W. C., and Palade, G. F., J. Biol. Chem., 172, 619 (1948).

7. LaDu, B. N., Gaudette, L., Trousof, N., and Brodie, B. B., J. Biol. Chem., 214, 741 (1955).

8. Mason, H. S., Science, 125, 1185 (1957).

9. Mueller, G. C., and Miller, J. A., J. Biol. Chem., 202, 579 (1953).

10. Nash, T., Biochem. J., 55, 416 (1953).