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Substrate Specificity of the Rabbit Lung Flavin-Containing Monooxygenase for Amines: Oxidation Products of Primary Alkylamines

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SUMMARY

Substrate activity of a flavin-containing monooxygenase isolated from rabbit lung microsomes has been examined with a number of primary, secondary, and tertiary amines. Of the secondary and tertiary amines tested, trifluoperazine, prochlorperazine, *N*, *N*-dimethyloctylamine, desmethylperazine, and *N*-methyloctylamine half-saturate the enzyme at concentrations less than 100 μM. Although the lung enzyme does not exhibit detectable substrate activity with primary arylamines, it catalyzes *N*-oxygenation of alkylamines to oximes. Studies on the mechanism for the oxidation of *n*-dodecylamine suggest that the amine is first oxidized to the hydroxylamine which is then further oxidized to the oxime. This interpretation is based on product identification, kinetic studies, and changes in the ratio of hydroxylamine to oxime formed as a function of initial substrate concentration. Kinetic constants calculated for the oxidation of *n*-dodecylamine and *n*-dodecylhydroxylamine indicate that the latter saturates the enzyme at a 100-fold lower concentration than that required for the parent amine, and the hydroxylamine is the dominant product only at saturating concentrations of the amine. The ratio of substrate-dependent NADPH and O_2 consumption and product formation (hydroxylamine + 2 × oxime) is approximately 1.0:0.9:0.7. Although the reason for the less than stoichiometric yield of products is not known, uncoupling of the enzyme by primary amines does not appear to be a major factor since substrate-dependent increase in H_2O_2 formation is never more than 3% of substrate-dependent O_2 consumption.

Flavin-containing monooxygenases (EC 1.14.13.8) purified to homogeneity from the hog (1), mouse (2), and rat (3) liver catalyze NADPH- and oxygen-dependent oxygenation of diverse types of drugs and other xenobiotic compounds that possess a nucleophilic heteroatom (4–6). The liver enzyme from different species appears similar in physical and catalytic properties, but an analogous monooxygenase purified to apparent homogeneity from rabbit lung microsomes (6, 7) is an immunologically distinct protein and differs significantly from the liver enzyme in stability and substrate specificity (6–9). Studies on enzyme intermediates indicate that the lung and liver enzymes are mechanistically similar in that both form an unusually stable hydroperoxyflavin intermediate. However, steric factors controlling access to the hydroperoxyflavin of the lung enzyme appear to be quite different from those of the liver enzyme and some of the better tertiary amine substrates for the liver enzyme show no detectable activity with the lung enzyme (7, 8).

There have been several reports that primary alkylamines stimulate NADPH oxidation (10) and oxygen reduction (9, 10), suggesting that primary amines may be substrates of the lung enzyme. This would be the first example of a qualitative difference in specificities between the lung and liver enzymes since none of the primary alkylamines tested are substrates for the liver monooxygenase. However, primary alkylamines are known positive effectors for the hog liver monooxygenase and partially uncouple NADPH-dependent reduction of oxygen through decomposition of the hydroperoxyflavin intermediate. If primary alkylamines only uncouple the lung enzyme, then differences between the lung and liver enzymes would be quantitative rather than qualitative. Although stimulation of NADPH oxidation by primary alkylamines in the presence of the lung enzyme is presumptive evidence for substrate activity, these measurements alone cannot distinguish between substrate oxygenation and enzyme uncoupling.

This report describes an investigation into the nature and mechanism for the oxidation of primary alkylamines catalyzed by the rabbit lung flavin-containing monooxygenase. The results demonstrate that primary alkylamines are sequentially monooxygenated to oximes through intermediate hydroxylamines and both steps are NADPH- and oxygen-dependent.

Experimental Procedures

Materials

Commercial reagents of the highest purity available were purchased from the firms listed. $NADP^+$, NADPH, glucose 6-phosphate, *Leucon-*

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ostoc mesenteroides glucose-6-phosphate dehydrogenase, catalase, and Tricine were from Sigma. *n*-Octylamine, *n*-dodecylamine, aniline, and organic solvents were obtained from Aldrich. *p*-Aminodiazobenzene was purchased from Eastman, benzidine was from Merck, and *N*-methyloctylamine was from K and K Laboratories. Trifluoperazine and prochlorperazine were supplied by Smith, Kline and Beckman. Desmethylperazine was a gift from Dr. Bill York (present address, Alcon Laboratories, Fort Worth, TX). Aniline was purified by redistillation and the commercial primary alkylamines were converted to hydrochloride salts and recrystallized at least three times from ethanol before use. All other commercial products were used without further purification.

n-Octylhydroxylamine, *n*-octyloxime, *n*-dodecylhydroxylamine, and *n*-dodecyloxime were synthesized by procedures described previously (11, 12). These compounds were recrystallized three times and residual solvent was removed under vacuum. The structures of the derivatives were verified by proton NMR spectroscopy (13) (Fig. 1).

The *cis* and *trans* isomers of *n*-dodecyloxime were separated by recrystallization. The *cis* isomer was quite insoluble in cold hexane and relatively pure crystals of this isomer were obtained when a hot saturated hexane solution containing a mixture of both isomers was slowly cooled. The remaining mother liquor was enriched about 90% in the *trans* isomer, but attempts to obtain stable preparations of this isomer were unsuccessful. Since *n*-dodecyloxime appears to slowly isomerize in the solvents employed, solutions of pure *cis* and enriched *trans* oxime were prepared just before use.

The flavin-containing monooxygenase was isolated from lung microsomes of pregnant rabbits by the method described earlier (8). The flavin adenine dinucleotide content of the enzyme preparations used in this study was approximately 14 nmol/mg of protein.

Methods

Assay of enzyme activity. Kinetic constants were calculated from substrate-dependent oxygen uptake at 37° in media containing 0.2 mM $NADP^+$, 1.5 mM glucose 6-phosphate, 1.0 IU of glucose-6-phosphate dehydrogenase, and 50 mM Tricine or Tris, pH 7.8, in a final volume of 2.0 ml. After 3–4 min for temperature equilibration, 0.5–1.0 nmol of monooxygenase in 5–10 μl of buffer was added through the capillary access port and the endogenous rate of oxygen reduction was recorded for 1–2 min. The amine substrate, dissolved in no more than 20 μl of water, was added and oxygen uptake was recorded for 2–10 min. Kinetic constants were calculated from initial reaction velocities at substrate concentrations above and below K_m. However, the constants for substrates with an apparent Km below 10 μM were estimated from oxygen uptake curves with limiting substrate by the method described previously (14).

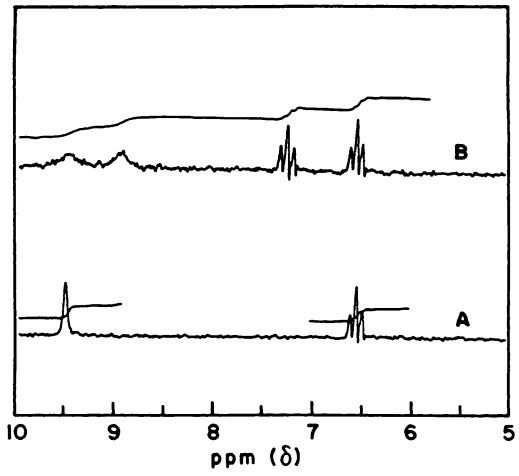

Fig. 1. Identification of the geometric isomers of *n*-dodecyloxime. Proton nuclear magnetic resonance spectra of *cis*-*n*-dodecyloxime (A) and an approximately equal mixture of *cis*- and *trans*-*n*-dodecyloxime (B) dissolved in deuterochloroform containing 1% trimethylsilane. The spectra were obtained on a Varian EM-390 at 90 MHz. The olefinic signals at δ 6.54 (triplet, 1H) and δ 7.26 (triplet, 1H) are characteristic of *cis* and *trans* alkyloximes, respectively. The hydroxyl proton signal can be observed at δ 9.50 (*cis*) and δ 8.94 (*trans*).

Product analysis. The products from the enzymic reaction mixtures were extracted into organic solvents and identified by chromatography beside reference compounds on thin layer silica gel plates (Eastman No. 13179). Solvent systems used for developing the chromatograms and R_f values calculated for reference compounds are given in Table 3. Compounds were detected by staining with iodine vapor or reaction with 1% ninhydrin in methanol containing 0.25% cadmium acetate (15). The relative concentration of oxime isomers separated on thin layer plates was estimated by comparison to synthetic standards of known concentrations.

The concentration of hydroxylamines in the enzyme reaction medium was determined by minor modifications of the method described earlier (16). Aliquots of 0.5–1.0 ml were taken at 0, 5, and 10 min and transferred to tubes containing 2.0 ml of ethanol. Insoluble material was removed by centrifugation and 0.4 ml of the clear supernatant fraction was mixed with 0.35 ml of 1.0 M sodium acetate (pH 4.5), 0.2 ml of 0.01 M 4,7-diphenyl-1,10-phenanthroline, and 0.05 ml of 0.01 M ferric nitrate. After 5 min at room temperature in the dark, 0.02 ml of 6.0 M phosphoric acid was added and the absorbance at 535 nm was measured. The concentration of hydroxylamine was calculated based on an absorptivity per reducing equivalent of 19.6 mM^{-1} cm^{-1} (16). The presence of alkylamines or alkyloximes did not interfere with the determination of hydroxylamines, and 5–100 μM *n*-octylhydroxylamine and *n*-dodecylhydroxylamine added to the reaction mixture were measured essentially quantitatively (±2%).

The concentration of *n*-dodecyloxime in enzymic reaction mixtures was measured by the following method, in which the oxime was first extracted into toluene, converted to the more stable nitrile, and then the concentration of the latter was estimated by gas chromatography. Aliquots of the reaction mixture taken after 0, 5, and 10 min incubation were transferred to tubes containing 0.2 ml of 1 M dibasic potassium phosphate and 0.6 ml of toluene. The contents were immediately mixed thoroughly, then briefly centrifuged to separate the phases, and 0.3 ml of the clear toluene extract was transferred to tubes containing 3 μl of acetic anhydride. The latter is necessary for quantitative thermal conversion of the oxime to the nitrile (17) during analysis by gas chromatography. The toluene extracts were analyzed with an analytical gas chromatograph equipped with a flame ionization detector and a 2000 × 0.4 cm column packed with 15% KOH-Carbowax 20 (0.75/30 on Chromosorb A). *n*-Dodecyloxime was quantitatively converted to the nitrile at an injection port temperature of 260° and *n*-dodecylnitrile (retention time 2.2 min) was completely resolved from the parent amine, hydroxylamine, and/or their decomposition products at a gas flow rate of 100 ml/min at 210°. Aliquots of the toluene extract (20–100 μl) were routinely used per analysis and the concentration of oxime was calculated from standard curves prepared with known concentrations of *n*-dodecyloxime dissolved in toluene/acetic anhydride (100:1). The oxime (50–1000 μM) added to the complete reaction mixture was estimated essentially quantitatively (96–98%) by this procedure. The autooxidation of *n*-dodecylhydroxylamine to the oxime was surprisingly slow under the conditions specified, and neither the hydroxylamine nor the parent amine interfered with the estimation of *n*-dodecyloxime.

The rate of H_2O_2 formation was estimated by measuring formaldehyde formed from the peroxidation of methanol in the presence of added catalase. Catalase (100 μg/ml) and methanol (100 mM) were added to the standard reaction mixture and formaldehyde was determined by the method of Nash (18) in aliquots of the reaction mixture deproteinized in 0.3 M trichloroacetic acid.

Results

Studies by Ohmiya and Mehendale (19) demonstrated that rabbit lung microsomes, unlike rat lung microsomes, did not

catalyze *N*-oxygenation of chlorpromazine. Subsequent work by Williams *et al.* (8) indicated that a number of the better tertiary amine substrates for the hog liver enzyme showed no detectable activity with the purified rabbit lung monooxygenase. However, the latter preparation catalyzed *N*-oxygenation of trifluoperazine, prochlorperazine, and *N*,*N*-dimethylaniline. We have confirmed these observations and, in addition, measured kinetic constants for the oxidation of these tertiary amines with the lung enzyme (Table 1). Whereas the K_m for *N*,*N*-dimethylaniline was higher than that of trifluoperazine, the velocities at saturating substrate concentrations are similar. However, V_{max} for prochlorperazine was consistently about 2 times that of trifluoperazine. The differences in V_{max} are surprising since these two tricyclic compounds are quite similar in structure. Unlike the hog liver enzyme for which V_{max} is relatively constant, it appears that subtle differences in substrate structure may influence V_{max} with the rabbit lung monooxygenase.

The secondary amines, desmethylperazine and *N*-methyloctylamine, also stimulate oxygen uptake and presumably are oxygenated to the corresponding hydroxylamines, although a detailed analysis of oxidation products was not carried out. The product expected from *N*-methyloctylamine, *N*-methyloctylhydroxylamine, was also an excellent substrate for the lung enzyme (Table 1). The oxidation of *N*-methyloctylhydroxylamine to the corresponding nitrone (20) followed by hydrolysis would lead to the formation of formaldehyde which was detected as one of the final reaction products (data not shown). This observation suggests that the reaction sequence for the oxidation of secondary amines by the lung enzyme is similar to that of the hog liver enzyme (4).

None of the primary arylamines tested showed detectable substrate activity with the lung enzyme (Table 2). However, all of the primary alkylamines tested stimulated NADPH oxidation and oxygen uptake and the kinetic constants for *n*-octylamine and *n*-dodecylamine were in agreement with those reported by Tynes *et al.* (9). In addition, the corresponding hydroxylamines also stimulated oxygen uptake, but these derivatives saturated the enzyme at much lower concentrations than did the parent amines. In contrast, the first stable oxidation products of hydroxylamines, the oximes, showed no detectable substrate activity (Table 2).

Analysis of toluene extracts of reaction media demonstrated that the lung enzyme catalyzed the oxidation of *n*-dodecylamine to two major products that comigrated with *n*-dodecylhydroxylamine and *n*-dodecyloxime in solvent systems II and IV (Table 3). The formation of products was dependent upon NADPH, enzyme, and incubation time. Nonenzymatic oxidation of *n*-dodecylamine could not be detected even after 30 min incubation in media lacking either NADPH or enzyme. The *cis* and *trans* isomers of *n*-dodecyloxime were readily resolved in solvents I, III, and V (Table 3), and, from the relative intensity of spots after staining with iodine, it appeared that the *cis* isomer predominated. The enzyme also catalyzed the oxidation of *n*-dodecylhydroxylamine to the oxime and visual inspection of the plates suggested that the relative amounts of *cis* and *trans* isomers appeared similar to that obtained from *n*-dode-

TABLE 1

Kinetic constants for oxidation of sec- and tert-amines catalyzed by the purified rabbit lung monooxygenase

The constants were calculated from initial rates of substrate-dependent oxygen uptake at 37° measured over a 100-fold range in substrate concentration. The reaction media contained 0.1 M Tricine, pH 8.4, the NADPH-generating system, and 0.23 μM rabbit lung flavin-containing monooxygenase.

Substrate	K_m	V_{max}
	μM	nmol/min, mg
N,*N*-Dimethylaniline	330	450
N,*N*-Dimethyloctylamine	46	450
Trifluoperazine	31	450
Prochlorperazine	3[a]	900
Chlorpromazine		NA[b]
Imipramine		NA
Chlorcyclizine		NA
Desmethylperazine	1000	900
N-Methyloctylamine	120	550
N-Methyloctylhydroxylamine	17	500

[a] Because velocities at substrate concentrations below K_m could not be measured accurately, this value was calculated from the change in slope of the oxygen uptake tracing by the method described in Ref. 14.

[b] NA, no activity. Substrate-dependent oxygen uptake could not be detected at concentrations of these compounds approaching saturation in the assay medium.

TABLE 2

Substrate specificity of the rabbit lung flavin-containing monooxygenase: Primary amines and derivatives

Kinetic constants were calculated from substrate-dependent oxygen uptake in reaction media identical to those given in Table 1.

Compound	K_m	V_{max}
	μM	nmol/min, mg
n-Octylamine	12,000	1,600
n-Dodecylamine	290	1,600
n-Octylhydroxylamine	70	600
n-Dodecylhydroxylamine	3	600
n-Octyloxime		NA[a]
n-Dodecyloxime		NA
Aniline		NA
Benzidine		NA
p-Aminodiazobenzene		NA
2-Napthylamine		NA

[a] NA, no activity. Substrate-dependent oxygen uptake could not be detected at concentrations of these compounds approaching saturation in the assay medium.

TABLE 3

Thin layer chromatographic properties of n-dodecylamine and its oxidation products

Authentic compounds dissolved in toluene were spotted on the silica gel plates (Eastman No. 13179) and developed in the solvent systems indicated: I, chloroform:isopropanol (95:5); II, isopropanol; III, chloroform:isopropanol:acetic acid (95:5:1); IV, isopropanol:ammonium hydroxide, 14 M (99:1); and V, dichloromethane.

	R_f values in solvent systems				
	I	II	III	IV	V
Standards					
n-Dodecylamine	0.04	0.05	0.04	0.30	0.00
n-Dodecylhydroxylamine	0.18	0.57	0.30	0.64	0.03
cis-*n*-Dodecyloxime	0.58	0.87	0.61	0.71	0.21
trans-*n*-Dodecyloxime	0.66	0.87	0.69	0.71	0.26
Enzymic reaction products from:[a]					
n-Dodecylamine	0.18	0.57	0.30	0.64	0.03
	0.58	0.87	0.61	0.71	0.21
	0.66[b]		0.69[b]		0.26[b]
n-Dodecylhydroxylamine	0.58	0.87	0.61	0.71	0.21
	0.66[b]		0.69[b]		0.26[b]

[a] Enzyme- and incubation time-dependent products extracted into toluene from the reaction mixture as described under Experimental Procedures.

[b] The apparent concentration of the *trans* isomer was never more than 10–15% of the *cis* isomer as judged by the intensity of the spot after exposure to iodine vapor.

cylamine. The oxidation of the hydroxylamine to the oxime was rapid only in the presence of NADPH and enzyme, although traces of oxime apparently formed by autooxidation of the hydroxylamine could be detected after 30 min incubation. Quantitative measurements of hydroxylamine and oxime formation from *n*-dodecylamine suggest that the ratio of products at 5 min incubation appears to be a function of initial amine concentration (Fig. 2). *n*-Dodecyloxime was the only product detected at concentrations of *n*-dodecylamine below K_m (Table 2), whereas at concentrations of the amine 10 times K_m, *n*-dodecylhydroxylamine was the major product.

The sum of hydroxylamine and oxime formed was consistently less than stoichiometric with oxygen uptake (Fig. 3) or NADPH oxidation (data not shown). The ratio of substrate-dependent oxygen uptake of NADPH oxidation was near 1 (0.9–0.95), but oxygen consumption was 25–30% greater than that required for product formation. Although *n*-dodecylamine slightly stimulated the rate of H_2O_2 generation (Fig. 3), the amount was not sufficient to account for the discrepancy in stoichiometry.

In contrast, oxygen uptake was less than stoichiometric with hydroxylamine consumption for the oxidation of the hydroxylamine to the oxime as indicated by a typical oxygen uptake tracing reproduced in Fig. 4. Since both hydroxylamine concentration and oxygen uptake can be measured with considerable precision, the 10–15% less oxygen reduced than hydroxylamine added indicated that the hydroxylamine was not quantitatively oxidized to the oxime. This was confirmed by analyzing for products in aliquots of the reaction mixture withdrawn 1 min after oxygen uptake had apparently returned to the initial endogenous rate (Fig. 4). In addition to the oxime, the extracts also contained a ninhydrin-positive component that comigrated with authentic *n*-dodecylamine. Although the concentration of the amine was not determined, its formation was dependent on

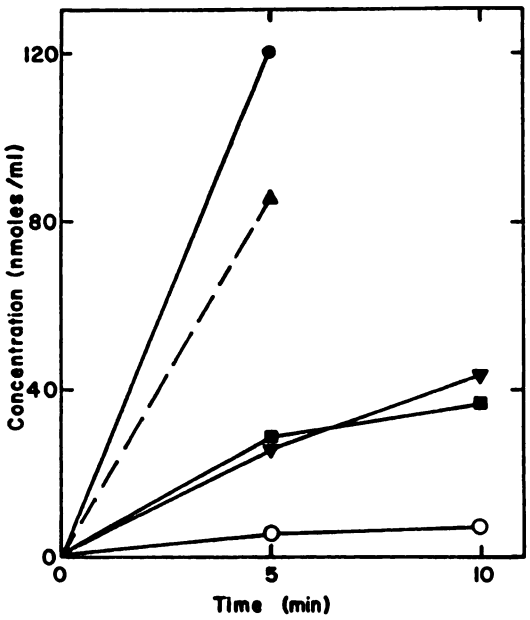

Fig. 3. Changes in rate of *n*-dodecyloxime and *n*-dodecylhydroxylamine formation as a function of reaction time. The reaction was carried out in the oxygraph vessel at 37° in media containing 0.1 M Tricine, pH 7.8, 100 mM methanol, catalase, the NADPH-generating system, and 0.7 μM rabbit lung enzyme in a volume of 3 ml. The reaction was started by adding *n*-dodecylamine (0.5 mM); aliquots were withdrawn for the zero time points, the vessel was closed, and the rate of oxygen uptake (●) was recorded for 5 min. The concentrations of *n*-dodecyloxime (▼) and *n*-dodecylhydroxylamine (■) were measured in aliquots withdrawn at 0, 5, and 10 min after addition of *n*-dodecylamine. The rate of H_2O_2 formation (○) was measured under identical conditions in separate experiments. Theoretical oxygen uptake required for formation of products measured is shown by – – – ▲.

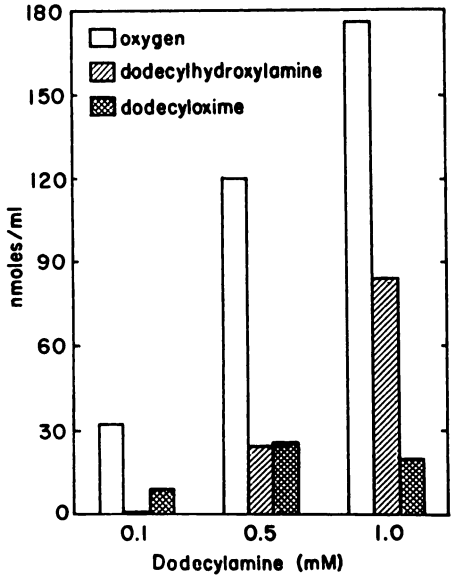

Fig. 2. Oxygen uptake and distribution of products as a function of *n*-dodecylamine concentration. The assay media contained 0.1 M Tris, pH 7.8, the NADPH-generating system, and 0.7 μM enzyme flavin. After a 4-min temperature equilibration the reaction was started by addition of *n*-dodecylamine to the concentrations listed. Aliquots withdrawn after 0- and 5-min incubation periods were analyzed for the products listed and oxygen uptake was determined polarigraphically as described under Experimental Procedures.

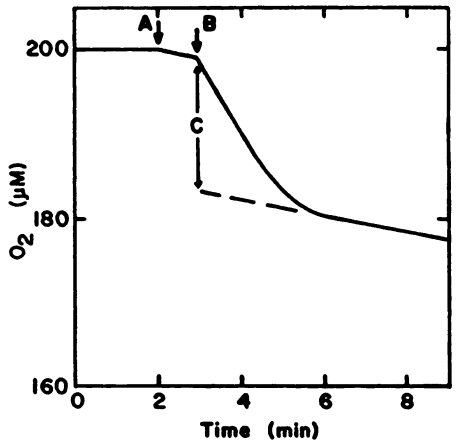

Fig. 4. Oxygen uptake with limiting *n*-dodecylhydroxylamine. The reaction was carried out at 37° in media identical to those listed in the legend to Fig. 3. Enzyme was added (A), and, after recording endogenous oxygen uptake for 1 min, 37 nmol of *n*-dodecylhydroxylamine in 5 μl of water were added (B) to the 2-ml reaction vessel through the capillary access port. Oxygen uptake was recorded until the rate returned to the initial endogenous rate. Substrate-dependent oxygen uptake of 16.1 nmol was determined from the delta oxygen decrease at C. The concentration of *n*-dodecylhydroxylamine in a solution prepared just before use was determined by the colorimetric method described under Experimental Procedures. The concentration was consistently 98–99% of the expected value based on the weight of the crystalline material.

both NADPH and enzyme which suggests that the flavoprotein may catalyze both oxidation and reduction of *n*-dodecylhydroxylamine.

Discussion

Previous reports (6–9) have shown that a flavin-containing monooxygenase purified to apparent homogeneity from rabbit lung microsomes is similar in molecular weight and catalytic mechanism to the hog liver enzyme but differs significantly in substrate specificity. The differences are both qualitative and quantitative, and some of the qualitative differences in substrate specificities with tertiary amines described earlier have been confirmed (Table 1). In addition, the data in Table 1 indicate that the substrate activity of trifluoperazine or prochlorperazine is not due solely to the piperazine moiety since chlorcyclizine, which also contains an *N*-methylpiperazine group, is not a substrate. Although the molecular basis for the lack of substrate activity of chlorcyclizine and chlorpromazine with the lung enzyme is not known, it would appear that the distance between the nucleophilic heteroatom and bulky groups on the side chain is a more important factor controlling access to the hydroperoxyflavin in the lung than in the liver enzyme.

The distinct substrate specificities of these monoooxygenases are most evident from studies with primary amines. Earlier reports have shown that primary alkylamines stimulated NADPH oxidation (9, 10) in the presence of the lung enzyme, and hydroxylamines have been detected in the reaction media (9), although at concentrations much less than stoichiometric with oxygen uptake. However, information presented in Figs. 2–4 clearly shows that *n*-dodecylamine is *N*-oxygenated to the oxime through the intermediate hydroxylamine as illustrated in Scheme 1. Both steps in the oxidation appear to be completely enzyme dependent. Although hydrogen peroxide generated by NADPH-dependent reduction of oxygen may nonenzymically oxidize primary amines in the reaction media, this apparently does not occur to a significant extent under the assay conditions since the addition of catalase did not have any effect on product formation (Fig. 3).

The major product in the enzyme-catalyzed oxidation of *n*-dodecylhydroxylamine appears to be the corresponding *cis* oxime. Although stereoselective oxidations catalyzed by the hog liver enzyme have been described (21), chemical synthesis of *n*-dodecyloxime produces a similar ratio of *cis* to *trans* products. These observations suggest that the hydroxylamine is enzymatically *N*-oxidized to the corresponding dihydroxylamine which is then dehydrated nonenzymatically in a reaction favoring the formation of the *cis* oxime (Scheme 1). However, the less than stoichiometric yield of amine oxidation products with respect to NADPH oxidized or oxygen reduced (Figs. 3 and 4) suggests that Scheme 1 is not a complete description of the enzyme-catalyzed reactions, and some other minor reaction is probably occurring. The nature of this postulated minor reaction in the overall oxidation of *n*-dodecylamine to *n*-dodecyloxime has not been fully established, but reactions initiated with synthetic *n*-dodecylhydroxylamine indicate that some of the hydroxylamine may be reduced to the parent amine. Although the enzyme-catalyzed reduction of the hydroxylamine has not been examined in detail, the reaction appears relatively slow and there is no question that, in the presence of oxygen, oxidation to the oxime is the major reaction (Fig. 4). However, if a fraction of the intermediate hydroxylamine is reduced as well as oxidized in reactions initiated with *n*-dodecylamine, oxygen uptake would be greater than expected from the formation of hydroxylamine plus oxime, and enzyme-dependent reduction of the hydroxylamine by NADPH may account for the observed descrepancies. Although this interpretation appears reasonable, a more detailed analysis of kinetic parameters for the reduction of the hydroxylamine is essential before this suggestion can be accepted or rejected.

Kinetic constants calculated for the oxidation of *n*-octylamine, *n*-dodecylamine, and their hydroxylamine derivatives indicate that the hydroxylamines are much better substrates for the purified lung enzyme than for the parent amine (Table 3), and at less than saturating concentrations, *n*-dodecylamine is converted to the oxime (Fig. 2). To what extent this would also occur in the intact rabbit lung is not known, but the kinetic constants indicate that, with time, primary alkylamines may be converted *in vivo* largely to the oximes. Since oximes are relatively nonpolar, they may require further metabolism for translocation and excretion. However, there is virtually no information on the toxicity, metabolism, or disposition of oximes, and further studies are required to determine whether the *n*-oxygenation of primary alkylamines in rabbit lung leads to detoxication or metabolic activation of primary alkylamines.

The lack of substrate activity of the primary arylamines tested (Table 3) is also difficult to explain. In general, arylamines are more easily oxidized than alkylamines, but the apparent lack of substrate activity of arylamines suggests that they are effectively excluded from the catalytic site on the enzyme. This difference is not due solely to differences in the size of the molecule since *N*,*N*-dimethylaniline or the perazine-substituted phenothiazines (Table 1) are similar to or larger in size than aniline or benzidine. Steric factors that exclude binding of primary arylamines are virtually unknown, but these results indicate that subtle differences in structure have a rather dramatic effect on the interaction of amines with the rabbit lung flavin-containing monooxygenase.

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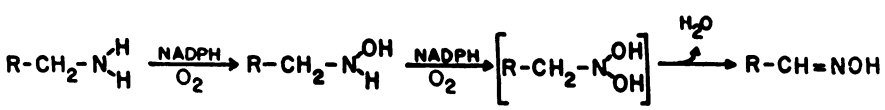

Scheme 1. Intermediates in the oxidation of primary amines catalyzed by the rabbit lung flavin-containing monooxygenase.

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