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Properties of Expressed and Native Flavin-Containing Monooxygenases: Evidence of Multiple Forms in Rabbit Liver and Lung

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SUMMARY

Our laboratory recently isolated and sequenced cDNAs encoding the microsomal flavin-containing monooxygenases (FMOs) from rabbit liver and rabbit lung. As a first step in understanding the molecular bases for the catalytic and physical differences between these enzymes, we have expressed them in COS-1 cells and compared the properties of the recombinant and native microsomal proteins. Microsomes from transfected cells were examined immunochemically by immunoblotting and catalytically by following methimazole oxidation in the presence and absence of various modulators. The expressed and native FMOs have the same mobilities in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the same responses to pH, sodium cholate, magnesium, and temperature, all of which serve to differentiate between the lung and liver enzymes. Analysis of methimazole metabolism in microsomes isolated from rabbit liver or lung showed biphasic kinetics, indicative of two or more enzymes taking part in the reaction. In contrast, the kinetics of methimazole oxidation catalyzed by the expressed FMOs were clearly linear and matched one of the phases observed with the native preparations. Chlorpromazine and imipramine, which are not substrates for the pulmonary FMO, were found to be competitive inhibitors of the high affinity reaction in pulmonary microsomes. These results, and others, indicate that both rabbit lung and liver contain more than one form of FMO.

The FMOs are microsomal xenobiotic-metabolizing enzymes that have been detected in liver, lung, and kidney, as well as other tissues, of all mammals examined. The FMOs catalyze the monooxygenation of numerous drugs, pesticides, and other xenobiotics, at nucleophilic sulfur, nitrogen, and phosphorous atoms (1, 2). Although metabolism by FMOs can generally be viewed as a first step in the process by which foreign chemicals are excreted, highly reactive products may be formed with some substrates (3). Rather than being excreted, such products may elicit toxic responses through covalent binding to cellular macromolecules or by some other mechanism. In contrast to numerous exogenous chemicals, only a few endogenous substrates for the FMOs are known. As a result, no physiological rationale for differences in the expression of multiple FMOs among various tissues is known.

Tissue-selective forms of FMO were initially hypothesized on the basis of differences between the activities of the enzymes in microsomal preparations from rabbit liver and lung (4, 5). Later, purification of the pulmonary and hepatic enzymes and comparison of their immunochemical, catalytic, and physical properties demonstrated more directly that the two FMOs are different proteins (6, 7). Now, it is known that these enzymes are products of distinct but related genes and have primary structures that are 56% identical (8). Although the enzymes show substantial divergence, which is consistent with their lack of immunochemical relatedness, a remarkable conservation of more general structural properties is evident from a comparison of their hydropathy indices and pyrophosphate-binding domains (8).

With the availability of cDNAs encoding the lung and liver FMOs, we have now expressed the enzymes in cultured cells and compared their properties in identical membrane preparations, something that the 100,000 × *g* particulate fractions from liver and lung are not (9). Expression in cell culture also avoids the two major problems associated with the study of purified FMOs, i.e., detergents introduced by the purification procedures and the possibility that the preparations contain more than one enzyme. The likelihood of the latter is reinforced by recent reports of multiple forms of hepatic FMO in guinea pig (10) and rabbit (11). The possibility of multiple enzymes provides an alternative to "substrate activation" (12, 13) as an explanation for the nonlinear kinetics associated with FMO-catalyzed reactions in hepatic microsomal fractions from mouse (14) and rat (12) and purified preparations from pig liver (13).

ABBREVIATIONS: FMO, flavin-containing monooxygenase; DTNB, dithionitrobenzoate.

By comparing the activities of preparations known to contain single enzymes with those of the native microsomal systems, we have been able to use expression in cell culture to gain some insight into the question of multiple forms of the enzyme in rabbit liver and lung.

Materials and Methods

Animals, tissues, and subcellular preparations. Adult, male, New Zealand White rabbits (Dutchland Farms, Denver, PA) were used. Tissues (liver, lungs, and kidneys) were removed, as quickly as possible, immediately after the animals were killed by suffocation with CO_2. Microsomal fractions were prepared by standard procedures (15). Microsomal fractions from COS-1 cells were prepared as described previously (16), except that the cells were disrupted by sonication for 15 sec (Cell Disruptor, Branson, Danbury, CT).

Construction of expression vectors and transfection. The cDNAs encoding the pulmonary and hepatic FMOs were recovered from Bluescript plasmid (pBS; Stratagene, La Jolla, CA) by digestion with restriction enzymes. Enzymes that limited the 5′ and 3′ flanking regions were selected to facilitate subcloning into the expression vector pCMV-5 (Dr. Mark Stinski, University of Iowa). The liver clone hFMO-1 was restricted with *Eco*RI and *Sspl*, and a fragment containing 55 5′ bases and 189 3′ bases was recovered. This fragment was subcloned into pCMV-5 digested with *Eco*RI and *Sma*I. The lung clones pFMO-1 and pFMO-2 (8) were originally recovered from pBS by restriction with *Cla*I and *Rsa*I and were subcloned back into pBS digested with *Cla*I and *Eco*RV. These constructs were then digested with *Cla*I and *Xba*I, and the isolated pFMO-1 and pFMO-2 fragments, each of which contained 28 5′ and 41 3′ bases, were subcloned into pCMV-5 restricted with the same enzymes. Proper orientation of each cDNA insert was confirmed by restriction analysis and by sequencing with primers complementary to regions flanking the multiple cloning site of pCMV-5 (17). Plasmid DNA was prepared for transfection by alkaline lysis followed by precipitation with polyethylene glycol (18). COS-1 cells were obtained from American Type Culture Collection and maintained as directed. The cells (approximately 80% confluent) were transfected by the DEAE-dextran procedure (19).

Immunoblotting procedures. Microsomal proteins from rabbit tissues and COS-1 cells (controls and 72 hr after transfection) were separated by electrophoresis on polyacrylamide gels (7.5%) in the presence of sodium dodecyl sulfate (20), transferred to nitrocellulose (BA-S 83; Schleicher and Schuell) (21), and immunostained according to the procedure described by Domin *et al.* (22). An antibody to a synthetic peptide, corresponding to amino acids 167–179 of the sequences derived from the liver and lung FMOs, was raised in goats and used for detection of both proteins. Cysteine was added to the carboxyl-terminal residue of the peptide to facilitate conjugation with keyhole limpet hemocyanin in the presence of *m*-maleimidobenzoyl-*N*-hydroxy succinamide ester (18, 23). The antiserum was used at a 1/1000 dilution.

Determination of FMO activity. FMO activity was determined with methimazole as the substrate, according to the method of Dixit and Roche (24). Microsomes in assay buffer (0.1 M tricine/KOH, pH 8.4, or 0.1 M potassium phosphate, pH 7.4), with 1 mM EDTA, 0.06 mM DTNB, 0.02 mM dithiothreitol, 6 mM KPO_4, and 0.1 mM NADPH, were added to sample and reference cuvettes and incubated at 37° for 30 sec before a base line of absorbance at 412 nm was recorded for a minimum of 2 min (Aminco DW-2a spectrophotometer). The reaction was initiated by the addition of methimazole to the sample cuvette. Inhibitors and other effectors were added as aqueous solutions to sample and reference cuvettes before addition of substrate. Treatment of microsomes with heat was done at 45° by incubation for 1–5 min, followed by rapid cooling on ice.

Kinetic analysis of methimazole metabolism. Kinetic constants were determined using rates measured over a 100-fold range of methimazole concentrations, by addition of increasing concentrations of methimazole to the sample cuvette. Data were analyzed with nonlinear regression analysis, using the ENZFITTER program.

Results

Kinetics of methimazole metabolism in rabbit pulmonary and hepatic microsomal preparations. Differences between the catalytic properties of the FMO enzymes in rabbit pulmonary and hepatic microsomal preparations can be seen clearly upon examination of the kinetics of methimazole metabolism (Fig. 1). The data, presented as Eadie-Hofstee plots, describe two reaction phases for each tissue. Although both liver and lung exhibited high affinity phases with K_m values slightly less than 5 μM, the K_m values for the low affinity phases differed by 10-fold: 36 μM for liver and 411 μM for lung (see Table 2). In liver the efficiency (V_{max}/K_m) of the high affinity reaction was less than 2 times that of the low affinity reaction, whereas in lung the difference was >5-fold in favor of the high affinity phase. Although the contribution of the high affinity phase to maximum activity was <20% in each case, its importance at low substrate concentrations, particularly in lung, was apparent. The relationships between rate and substrate concentration observed here and elsewhere (12–14) for FMO-catalyzed reactions can be explained by complex properties of a single enzyme or by the combined simple properties of two or

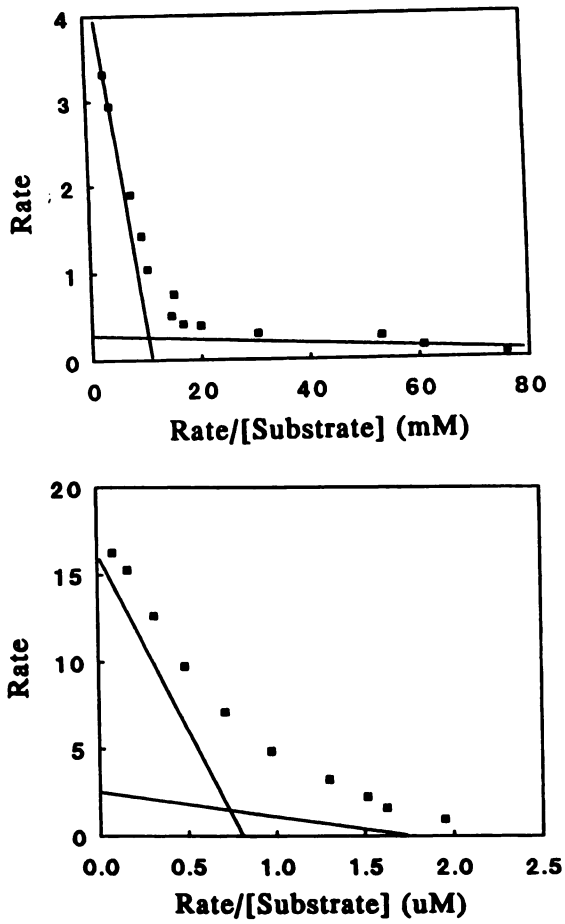

Fig. 1. Hofstee plots of methimazole oxidation in microsomal preparations from rabbit lung and liver. A, Pulmonary microsomes; B, hepatic microsomes. Reaction was monitored in 0.1 M tricineKOH buffer, pH 8.4, using ~50 μg/ml microsomal protein. The rate is nmol of product formed/min/mg of protein.

more enzymes. We have addressed this issue by examining the behavior of the lung and liver FMO enzymes expressed in COS-1 cells.

Expression of lung and liver FMOs in COS-1 cells. Cultured COS-1 cells were transfected with pCMV-5 containing cDNAs encoding the pulmonary or hepatic FMOs. Seventy-two hours after transfection, cells were harvested and the microsomal fractions were isolated. Microsomal proteins were separated on polyacrylamide gels, transferred to nitrocellulose, reacted with a polyclonal antibody to a synthetic peptide common to rabbit liver and lung FMOs (see Materials and Methods for details), and stained with diaminobenzidine (Fig. 2). The expressed pulmonary variants (COS-Lg1 from pFMO-1 and COS-Lg2 from pFMO-2) were both detected, but as proteins with slightly different mobilities (Fig. 2, *lanes 2* and *3*). When the COS-Lg1 and COS-Lg2 samples were mixed (Fig. 2, *lane 5*), two bands of protein were detected, the mobilities of which coincided with those from rabbit pulmonary microsomal samples (Fig. 2, *lane 4*, shows a phenotype that contains both Lg1 and Lg2; two phenotypes not shown contain either Lg1 or Lg2).[1] The expressed liver enzyme (COS-Lv from hFMO-1) was detected as a single band (Fig. 2, *lane 6*), having the same mobility as the rabbit hepatic microsomal enzyme (Fig. 2, *lane 7*). No proteins were detected in microsomal samples from control COS-1 cells, either with the peptide antibody (Fig. 2, *lane 1*) or with polyclonal antibodies to the pig liver or rabbit lung enzymes (data not shown). In order to establish the utility of the recombinant proteins for investigation of the catalytic properties of the FMOs, several general properties of the expressed enzymes were examined and compared with those of the native enzymes.

Activities of expressed FMOs and modulation by chemical effectors. The catalytic activities of the expressed FMOs were examined with methimazole, a substrate that meets three important criteria, i.e., it can be used over a wide range of concentrations, its metabolism can be assessed by a sensitive direct assay, and metabolism catalyzed by cytochrome P-450 is expected to be negligible (25). Microsomes prepared from transfected COS-1 cells (COS-Lg1, COS-Lg2, and COS-Lv microsomes) catalyzed the metabolism of methimazole at rates from 0.5 to 1.5 nmol/min/mg. Microsomes from control COS-1 cells had no detectable activity. The effects of magnesium (100 mM $MgCl_2$, *n*-octylamine (3 mM), and sodium cholate (1%), three known effectors of the FMO, on COS-Lg1,[2] COS-Lv, hepatic, and pulmonary microsomal activities are shown in Table 1 and Fig. 3. The activities of the hepatic and COS-Lv preparations were decreased (~40%) by magnesium and markedly decreased by cholate (~90%), whereas those of pulmonary and COS-Lg1 preparations were increased almost 2-fold by either treatment. Addition of *n*-octylamine slightly increased the activities of all four preparations, except for COS-Lg1.

pH optima and temperature sensitivities of the expressed and native FMOs. Because the rabbit pulmonary and hepatic FMOs have different pH optima and temperature sensitivities (7, 14, 26), these properties were examined with the expressed enzymes. The metabolism of methimazole, in COS-Lg1 and COS-Lv preparations, as a function of pH is shown in Fig. 4. These results, showing the pH optimum for COS-Lg1 to be about 1 unit higher than for COS-Lv, are very similar to those observed with the native microsomal enzymes (14). Initially, the reported difference between the effects of heat on the hepatic and pulmonary FMOs (26) was not observed with the expressed enzymes, both of which were found to be

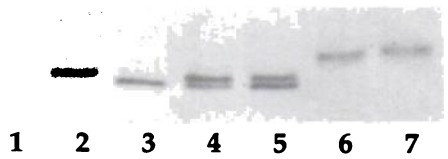

Fig. 2. Immunoblot of microsomal preparations from pCMV-Lg (COS-Lg)-and pCMV-Lv (COS-Lv)-transfected COS-1 cells and rabbit liver and lung. *Lane 1*, 200 μg of mock-transfected COS-1 cells; *lane 2*, 10 μg of COS-Lg2; *lane 3*, 10 μg of COS-Lg1; *lane 4*, 2 μg of rabbit lung; *lane 5*, 10 μg each of COS-Lg2 and COS-Lg1; *lane 6*, 10 μg of rabbit liver; *lane 7*, 50 μg of COS-Lv. Microsomal proteins were separated on a 7.5% polyacrylamide/sodium dodecyl sulfate gel, transferred to a nitrocellulose sheet, and reacted sequentially with an antibody to a synthetic peptide common to rabbit liver and lung FMO, with rabbit anti-goat IgG, and with peroxidase-anti-peroxidase, followed by staining with diaminobenzidine.

TABLE 1

Effects of magnesium and *n*-octylamine on the activities of native and expressed microsomal FMOs

Data are expressed as percentage of control activity and represent the average of activities determined using microsomes isolated from at least two individual animals or two independent transfections. Assays were done in 0.1 M tricine/KOH, pH 8.4, using 1 mM methimazole with rabbit liver and COS-Lv microsomes and 2 mM methimazole with rabbit lung and COS-Lg1 microsomes. Rates were measured 5 min after addition of methimazole to the sample cuvette.

Addition[a]	FMO activity			
	Liver		Lung	
	Rabbit	COS-Lv	Rabbit	COS-Lg1
	% of control			
100 mM $MgCl_2$	52, 69	58, 64	191 ± 17	200 ± 15
3 mM *n*-Octylamine	121, 135	141, 156	131, 128	98, 102

[a] Compounds were added to sample and reference cuvettes before addition of substrate to sample cuvette.

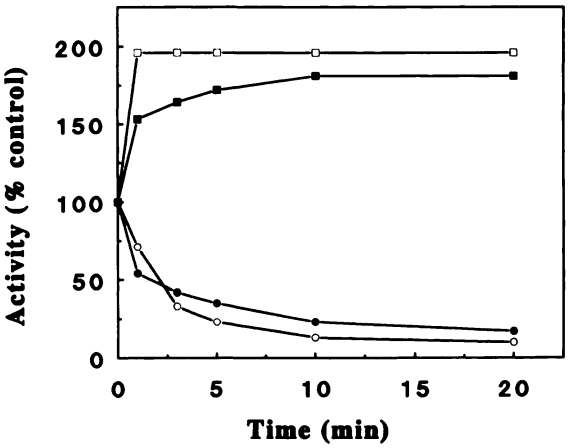

Fig. 3. Stability of native and recombinant FMOs in 1% sodium cholate. Activity was measured in 0.1 M tricineKOH, pH 8.4, using the methimazole/DTNB assay. Methimazole concentration was 1 mM with COS-Lv (○) and liver microsomes (●) and 2 mM with COS-Lg1 (□) and lung microsomes (■). Preparations were treated with sodium cholate (1%) for the times indicated. The 100% control value is the rate at time zero.

[1] Our previous report (29) of three bands associated with the pulmonary FMO is incorrect; one of the bands appears to have been an artifact of electrophoresis.

[2] In most cases, detailed experiments were not carried out with COS-Lg2. However, in preliminary experiments no differences between COS-Lg1 and COS-Lg2, aside from their mobilities, were noted.

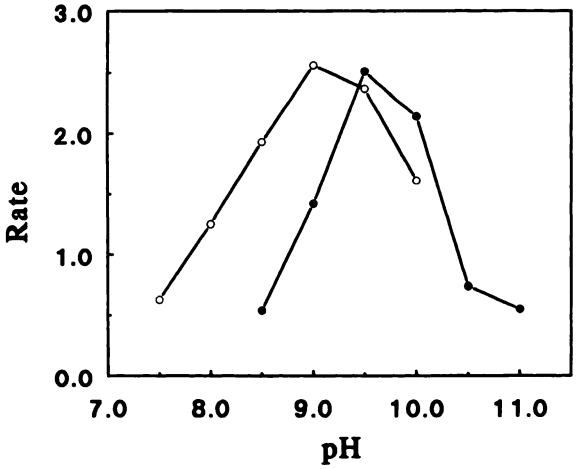

Fig. 4. Effect of pH on the activity of microsomes isolated from COS-1 cells transfected with pCMV-Lv or pCMV-Lg. Activity was measured using the methimazole/DTNB assay. Tricine buffer (0.1 M) was used with COS-Lv microsomes (○), and glycine buffer (0.1 M) was used with COS-Lg microsomes (●). The pH was adjusted with KOH. Methimazole concentration was 1 mM for COS-Lv microsomes and 2 mM for COS-Lg1 microsomes. The rate is nmol of product formed/min/mg of protein.

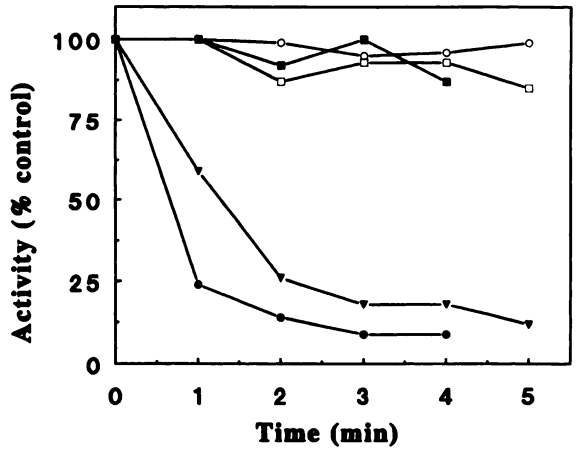

Fig. 5. Effect of temperature on the activity of native and recombinant FMOs. Microsomes were heated at 45° for the specified time and then placed on ice before measurement of methimazole oxidation in 0.1 M tricineKOH, pH 8.4. Methimazole concentration was 1 mM with unwashed COS-Lv (○), washed COS-Lv (▼), and liver microsomes (●) and 2 mM with COS-Lg1 (□) and lung microsomes (■).

stable (Fig. 5). This discrepancy was resolved by a single washing of the COS-Lv microsomes in homogenization buffer, followed by centrifugation for 15 min at 100,000 × *g*. With washed COS-Lv microsomes, 80% of the FMO activity was lost after 5 min at 45° (Fig. 5). The stability of the expressed pulmonary enzyme was retained after washing of COS-Lg1 microsomes (data not shown). With the demonstration that the recombinant FMOs retain the same general characteristics as the native enzymes, we next examined the kinetic behavior of the expressed proteins.

Kinetics of methimazole metabolism catalyzed by COS-Lv and COS-Lg1. The relationships between substrate concentrations and reaction rates for the oxidation of methimazole catalyzed by COS-Lg1 and COS-Lv microsomal preparations are shown in Fig. 6. The data, transformed by the method of Eadie-Hofstee, were clearly linear over the 100-fold substrate concentration ranges used. The K_m values for the

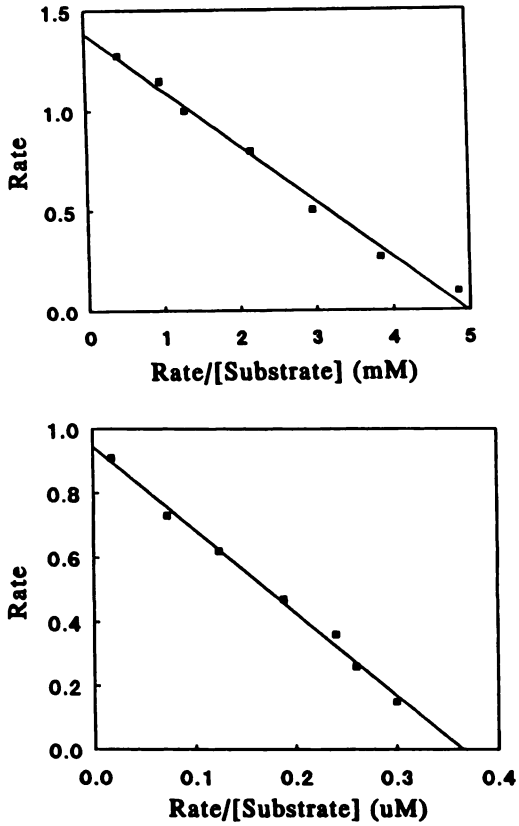

Fig. 6. Hofstee plots of methimazole oxidation in microsomal preparations from COS-1 cells transfected with pCMV-Lg or pCMV-Lv. A, COS-Lg1 microsomes; B, COS-Lv microsomes. Reaction was monitored in 0.1 M tricine/KOH buffer, pH 8.4, using ~200 μg/ml microsomal protein. The rate is nmol of product formed/min/mg of protein.

TABLE 2

K_m values for metabolism of methimazole catalyzed by FMOs present in microsomal preparations from liver, lung, and COS-1 cells transfected with pCMV-Lg1 or pCMV-Lv

Relative affinity	K_m			
	Lung[a]	Liver[b]	COS-Lg1[c]	COS-Lv[c]
		μM		
High	3.9 ± 1.9	3.3 ± 1.4		2.8, 4.7
Moderate		36 ± 11		
Low	411 ± 50		300, 330	

[a] Mean ± standard deviation for results obtained with preparations from four individual rabbits.
[b] Mean ± standard deviation for results obtained with preparations from three individual rabbits.
[c] Results obtained with two independent pCVM-Lg1 and pCMV-Lv transfections.

COS-Lg1 and COS-Lv systems were found to differ by 2 orders of magnitude: 3.8 μM for liver and 315 μM for lung. A comparison of these values with those obtained for the pulmonary and hepatic microsomal preparations is shown in Table 2.

Effects of alternative substrates on the activities of the expressed and native FMOs. Chlorpromazine and imipramine, substrates for the hepatic but not the pulmonary FMO, and thiourea, a substrate for both forms of FMO, were tested for their abilities to modulate the metabolism of methimazole catalyzed by COS-Lg1, COS-Lv, hepatic, and pulmonary microsomal preparations (Fig. 7). A fourth compound, prochlorperazine, which has been reported to be a low-K_m substrate for both the pulmonary and hepatic enzymes (27, 28), was also

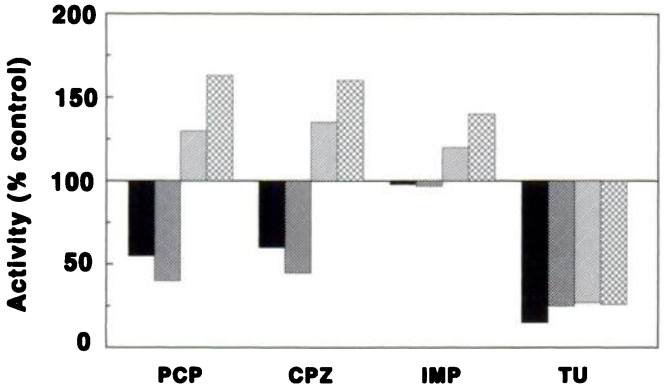

Fig. 7. Effects of various chemicals on the rate of methimazole oxidation catalyzed by native and recombinant FMOs. The concentration of methimazole used with rabbit liver (■) and COS-Lv (▩) microsomes was 30 μM and the concentration used with rabbit lung (▨) and COS-Lg1 (bX) microsomes was 2 mM. Inhibitor (*PCP*, prochlorperazine; *CPZ*, chlorpromazine; *IMP*, imipramine; *TU*, thiourea) (100 μM each) was added as an aqueous solution to sample and reference cuvettes before addition of substrate to the sample cuvette. Buffer was 0.1 mM potassium phosphate, pH 7.4. Values from COS microsomes represent the average of three independent transfections, and values from tissue microsomes represent the average of preparations from three individual animals. In each instance, the standard deviation was <10% of the mean.

tested. Each compound was used at a concentration of 100 μM, with methimazole concentrations of 30 μM for the hepatic and 2 mM for the pulmonary systems (7–10 times the estimated K_m values). Under these conditions, only thiourea inhibited (60–70%) the activities of all four preparations. Prochlorperazine and chlorpromazine inhibited both the native and expressed hepatic enzymes, as expected. However, inhibition by either compound was about 20% greater (67 versus 43% and 52 versus 30%) with the expressed enzyme. Imipramine, at the stated conditions, had no effect on the native or expressed hepatic systems but was an effective inhibitor of both when either its concentration was increased or the substrate concentration was decreased (data not shown). Both imipramine and chlorpromazine increased the activities of the native and expressed pulmonary systems. Imipramine was more effective than chlorpromazine in this regard, and the effects of both compounds were about twice as great with the expressed, compared with the native, preparations. Prochlorperazine, contrary to our expectations, also increased the activities of the native and expressed pulmonary enzymes. Again, the increase observed with the expressed enzyme was about twice that seen with the native preparation. This differential result was explored further by investigating the effects of chlorpromazine (100 and 500 μM) and imipramine (200 μM and 1 mM) on the high affinity phase of the pulmonary microsomal reaction. In contrast to the stimulation observed when the substrate concentration was 2 mM (see above), competitive inhibition of methimazole at low concentrations (5 to 40 μM) was observed with both chlorpromazine and imipramine (Fig. 8).

The possible involvement of cytochrome P-450 in the high affinity pulmonary phase and the low affinity hepatic phase was checked by inhibition with antibodies to NADPH cytochrome P-450 reductase. No alteration of reaction rates was observed at any concentration of methimazole, with antibody concentrations sufficient to inhibit cytochrome *c* reduction and cytochrome P-450-catalyzed reactions by >90% (data not shown).

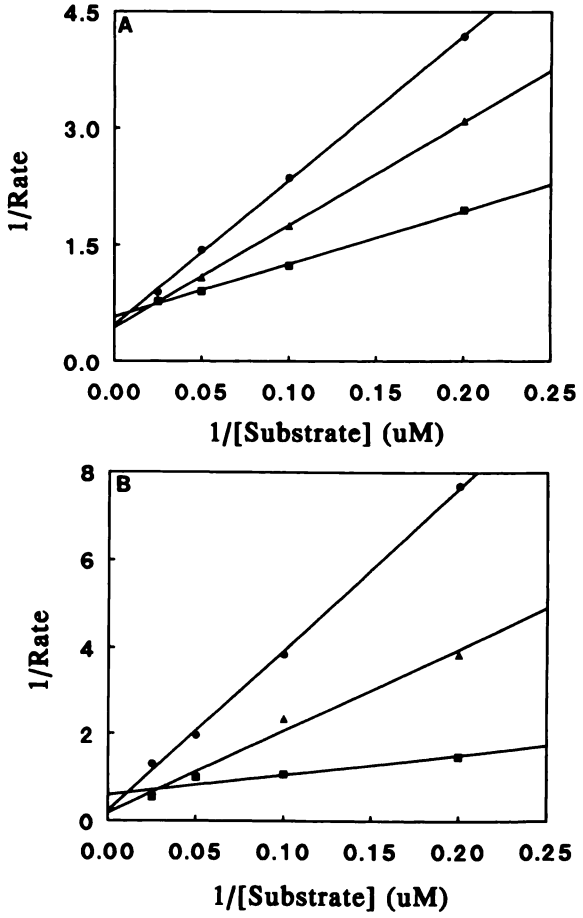

Fig. 8. Double-reciprocal plots of methimazole oxidation in rabbit pulmonary microsomal preparations in the presence of chlorpromazine and imipramine. A, Effect of imipramine, at 0 μM (■), 100 μM (●), or 500 μM (▲); B, effect of chlorpromazine, at 0 μM (■), 200 μM (●), or 1 mM (▲). The substrate concentrations were 5, 10, 20, and 40 μM methimazole. Reactions with chlorpromazine were done in 0.1 M potassium phosphate buffer, pH 7.4, and reactions with imipramine were done in 0.1 M tricineKOH, pH 8.4.

Discussion

The cDNAs encoding the hepatic and pulmonary isozymes of the FMO have been expressed in cultured COS-1 cells, and the recombinant proteins have been analyzed immunochemically, catalytically, and kinetically. The results, which show that the recombinant and native lung or liver FMOs are similar, indicate that the unique characteristics of the two enzymes are intrinsic and are not related to other differences between the pulmonary and hepatic microsomal fractions. Properties shared by the native and expressed enzymes include their responses to sodium cholate, temperature, pH, and magnesium and their mobilities in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Initially, a difference between the recombinant and native proteins was suggested by the thermostability of the hepatic enzyme in COS-1 microsomal preparations. However, it was found that the lability of the enzyme was restored when the microsomes were washed, likely due to the removal of NADPH or some other ligand of the type known to protect the hepatic enzyme against the effects of elevated temperature (26). The stability imparted to unwashed COS-1 preparations was not apparent when hepatic microsomes were examined.

Although most properties of the expressed and native en-

zymes were found to be similar, examination of the kinetics of methimazole metabolism revealed major differences with both the liver and lung systems. Kinetics with the expressed enzymes are clearly linear, and those with the native microsomal preparations are clearly nonlinear. In both cases, the kinetics of the expressed enzyme correspond to the kinetics of one of the phases observed with the native microsomal preparations, the high affinity phase with the liver and the low affinity phase with the lung.

Previously, it has been suggested that nonlinear kinetics for oxidations catalyzed by mouse (14) and rat (12) hepatic microsomal FMOs and purified pig liver FMO (13) result from enzyme activation caused by substrate association with an "activator binding site" (12, 13). Given the properties of the individual enzymes expressed in COS-1 cells, it seems more likely that the observed complex kinetics actually reflect the presence of two or more FMO enzymes in the preparations examined. The differential effects that some modulators have on the expressed and native systems support this conclusion. Inhibition of hepatic FMO activity by chlorpromazine and prochlorperazine is much greater with microsomes from COS-Lv than from liver, which is indicative of an FMO enzyme in liver for which chlorpromazine and prochlorperazine are not substrates. In a similar fashion, the greater activation of COS-Lg1, compared with pulmonary, microsomal preparations by imipramine, chlorpromazine, and prochlorperazine suggests that a pulmonary form of the enzyme either is not activated or is actually inhibited by these compounds. Competitive inhibition of methimazole metabolism by imipramine and chlorpromazine at low substrate concentrations indicates that these compounds are actually metabolized by the high affinity FMO in lung.

The similarity between the K_m of the expressed hepatic enzyme and that of the high affinity phase observed with pulmonary microsomes suggests that the high affinity phase of the pulmonary activity might be due to expression of some liver enzyme in lung. Inhibition of both the COS-Lv and the high affinity pulmonary activity reactions by imipramine and chlorpromazine is consistent with this possibility. However, although hepatic FMO is present in the lungs of pigs, guinea pigs, rats, mice, and hamsters (13, 29), neither protein nor mRNA for the rabbit hepatic enzyme has been detected in rabbit lung (6–8, 29). Similar findings indicate that the pulmonary FMO is not expressed in liver, a possibility that is inconsistent with the kinetic evidence in any case.

Recently, reports of multiple forms of hepatic FMO in guinea pig (10) and rabbit (11) have been published. Differences between mobilities, substrate specificities, peptide maps, and absolute spectra of two proteins purified from guinea pig liver were seen as evidence for multiple FMOs. However, no evidence for the existence of these proteins in microsomal preparations was obtained, and the results presented could have resulted from alteration of a single enzyme by proteolysis during purification. In contrast, the results of Ozols (11) cannot be explained by some posttranslational event. The complete sequence of one of the two proteins isolated (30) is nearly identical to that derived previously from cDNA encoding the rabbit liver FMO (8), whereas a partial sequence of the second, although clearly related, differs significantly (11). Also, we have presented preliminary results concerning an additional form of rabbit liver FMO that is equally related to the known hepatic and pulmonary forms but is not the second form described by Ozols (31). The individual contributions of these enzymes to the kinetic complexities observed with rabbit hepatic microsomes remain to be determined.

Although most of our results with the native enzymes agree with those published previously (4–7, 26), our findings with prochlorperazine do not. This drug was found to activate pulmonary methimazole metabolism, an unexpected result because the concentration used (100 μM) was 30-fold greater than its reported K_m as a substrate for the rabbit pulmonary FMO (27). Inhibition of COS-Lg1 activity by prochlorperazine (100 μM) could be observed, but only when the methimazole concentration was lowered to less than its K_m. This indicates that the K_m for prochlorperazine, if it is actually a substrate for the lung enzyme, is much higher than reported. The results of Poulsen *et al.* (27), who measured prochlorperazine-initiated oxygen consumption, could have been compromised by an uncoupling reaction and may not reflect substrate metabolism. It is of interest that imipramine and chlorpromazine, two compounds that are not substrates for the lung FMO, also activite the metabolism of methimazole in pulmonary and COS-Lg1 microsomal preparations.

We have expressed the rabbit liver and lung FMOs in COS-1 cells and shown that many of the properties of the recombinant and native proteins are similar. Kinetic comparisons of the two systems have led to the conclusion that multiple FMO enzymes are present in liver and lung. Our results demonstrate the utility of the COS-1 system for characterizing FMO enzymes under conditions where a single FMO gene product is known to be present. Future studies will focus on relationships between the structures and functions of the rabbit liver and lung FMOs through the use of site-directed mutagenesis, deletion analysis, and construction of chimeric proteins.

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