The Flavin-containing Monoxygenase Enzymes Expressed in Rabbit Lung and Lung Are Products of Related but Distinctly Different Genes*

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Preparations of mRNA isolated from rabbit lung and liver were used in the construction of libraries that were screened for cDNAs encoding the pulmonary or hepatic isozyme of the flavin-containing monoxygenase. The hepatic library was screened with cDNA encoding the flavin-containing monoxygenase expressed in pig liver, and a clone containing a 2.0-kilobase insert was detected and isolated. This cDNA insert encoded a protein of 553 amino acids with a primary structure 87% identical to that of the pig flavin-containing monoxygenase. The pulmonary library was screened with polyclonal antibodies to the flavin-containing monoxygenase. The pulmonary library was screened with cDNA encoding the flavin-containing monoxygenase expressed in rabbit lung, and a clone containing a 2.6-kilobase insert was detected and isolated. Although the protein encoded by this insert also contained 553 amino acids, its primary sequence was only 56% identical to that of the liver enzyme. The sequences of several peptides obtained by digestion of the purified rabbit pulmonary flavin-containing monoxygenase with trypsin matched exactly with sequences derived from the cDNA structure. Tissue-specific distribution of mRNA for the hepatic and pulmonary isozymes of the flavin-containing monoxygenase was consistent with the distribution of protein, an indication that expression of flavin-containing monoxygenase is controlled at the level of transcription. Analysis of genomic DNA indicates that both the hepatic and pulmonary enzymes may be products of single genes.

The toxic effects of a wide variety of environmental chemicals can be highly selective with respect to species, tissue, and cell type. Most of these chemicals must undergo oxidative metabolism before their reactivities can be expressed, and the distribution of the enzymes catalyzing this "activation" may contribute to the biological localization of toxic responses. The lung is a good example of a tissue that is selectively damaged by a number of toxic agents (1). Within the lung, the nonciliated bronchiolar (Clara) cell is particularly vulnerable (2), due likely to the relative abundance of specific isozymes of cytochrome P-450 (3). This association has been most clearly demonstrated in the case of the cell-selective toxic effects of certain substituted furans, such as 4-ipomeanol (4). Identical enzymes are also expressed in liver (5, 6) but at concentrations that are apparently low enough to preclude a toxic response in hepatocytes.

An additional enzyme of possible importance in differences between the responses of lung and liver to some toxic agents is the flavin-containing monoxygenase (FMO1, EC 1.14.-13.8), an enzyme that catalyzes the oxidation of numerous nitrogen-, sulfur-, and phosphorus-containing drugs, pesticides, and industrial chemicals (7, 8). As with cytochrome P-450, some reactions catalyzed by FMO result in the formation of reactive intermediates that are potentially toxic (9). The function of the FMO with respect to endogenous substrates is not known but may involve maintenance of the cellular thiol to disulfide ratio by oxidation of cysteamine to cystamine (10).

The majority of what is known about the molecular characteristics of the FMO comes from studies of the enzyme purified from pig liver. However, expression of distinct isozymes in liver and lung has been described recently. This possibility was first noted with the discovery that FMO activity in detergent-solubilized preparations from rabbit lung but not from liver could be stimulated by Hg++ (11). More substantial evidence was in a subsequent report that imipramine, which is metabolized by the FMO in liver, does not undergo N-oxidation in rabbit pulmonary microsomal preparations that are active with other FMO substrates (12). Finally, characterization of the FMO purified from rabbit lung provided direct evidence of major differences between the "hepatic" and "pulmonary" forms of the enzyme (13, 14).

The extent to which the structures of the pulmonary and hepatic forms of the FMO differ is suggested by their lack of immunochromatographic reactivity (13, 14), the stability of the pulmonary enzyme when subjected to temperatures or concentrations of ionic detergents that denature the hepatic enzyme (13), and the ability of the pulmonary enzyme to metabolize several primary arylamines that are not substrates for the hepatic enzyme (15, 16). The molecular bases for these differences are not understood, primarily because of a lack of information about the structures of these proteins. Recently, however, we have derived the primary sequence of the FMO expressed in pig liver (17), and we now report the primary sequences of the FMO isozymes expressed in rabbit lung and liver.

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1 The abbreviations used are: FMO, flavin-containing monoxygenase; kb, kilobases; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography.
Zealand White rabbits (Dutchland Farms, Denver, PA) were used. Tissues (liver, lungs, and kidneys) were removed immediately after the animals were killed by suffocation with CO₂. RNA and DNA were isolated from tissues that were frozen in liquid nitrogen and stored at -70 °C, and microsomal fractions were prepared from fresh tissue by standard procedures (18).

**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 immediately after the animals were killed by suffocation with CO₂. RNA and DNA were isolated from tissues that were frozen in liquid nitrogen and stored at -70 °C, and microsomal fractions were prepared from fresh tissue by standard procedures (18).

Construction and Screening of cDNA Libraries—Preparations of hepatic and pulmonary mRNA were isolated by a modification (6) of the methods of Chirgwin et al. (19) and Glisin et al. (20). A liver cDNA library was constructed in Agt11 (6) and screened by plaque hybridization with nick-translated cDNA encoding the FMO expressed in pig liver (17). Similarly, a pulmonary cDNA library was constructed in Azap II (Stratagene, La Jolla, CA) after selection of cDNA species between 1.3 and 2.6 kb. The pulmonary library was screened with antibodies to the pulmonary FMO (14). Recombinant clones were identified by detection (21, 22) of antigen β-galactosidase fusion protein following transfer of plaques to nitrocellulose (23). Seven clones were purified and isolated as subclones in Bluescript plasmid (pBS; Stratagene).

Nucleotide Sequencing—Purified inserts from pulmonary (pFMO-1) or hepatic (hFMO-1) clones were fragmented by sonication (24) or restriction (25) and sequenced by the dideoxy chain termination method (26, 27). Additional pulmonary clones were sequenced using oligonucleotide primers complementary to the T3 and T7 promoter regions flanking the inserts (28). The sequence data were analyzed with the sequence analysis software package from the University of Wisconsin Computer Group (29), and gap alignments were constructed with the algorithm developed by Wilbur and Lipman (30).

Analysis of mRNA and Genomic DNA—Messenger RNA purified from liver, lung, and kidney was separated by electrophoresis in agarose (FMC BioProducts, Rockland, ME) gels (1%) containing 100 ng/ml salmon sperm DNA, 1% SDS, and 50% formamide. Hybridized blots were washed twice for 2 h at 42 °C and then reacted with full-length, 5' and 3' PFM0-1 probes. The reactions were carried out overnight at 55 °C in hybridization solution (6 × SSC, 50 μg/ml salmon sperm DNA, 1% SDS, and 50% formamide). Hybridized blots were washed twice for 30 min at 65 °C in 0.1 × SSC containing 0.1% SDS and then subjected to autoradiography.

Sequencing of FMO Purified from Rabbit Lung—The FMO expressed in rabbit lung was purified by the method of Tynes et al. (14). Purified protein (2 nmol) in elution buffer (10 mM phosphate, pH 7.6, 10 mM EDTA, 0.1 mM diithiothreitol, 20% glycerol, 0.25% Emulgen 911, and 1 mM NADPH) was placed in a filter-centrifuge tube (Amicon Centricon-30) and washed sequentially with 0.015 M Tris (pH 8.6), 5 mM guanidinium HCl, and 0.015 M Tris + 5 mM guanidinium HCl. The protein was then treated with 0.5 mg of diithiothreitol, incubated for 1 h at 37 °C, treated with 1.5 mg of iodoacetic acid, and finally incubated in the dark at room temperature for 1 h. The alkylated protein was washed with 0.015 M Tris, pH 8.6, resuspended in 2% NH₄HCO₃, and digested with trypsin (10 μg) at 37 °C for 1 h, after which additional trypsin (5 μg) was added and the incubation continued for 30 min. The resulting peptides were separated on a 300A C-18 column using the following elution scheme: 95% H₂O, 10% acetonitrile for 1 min followed by a linear change to 50% H₂O, 50% ACN over 59 min. The flow rate was 0.9 ml/min, and peptide elution was monitored at 214 and 254 nm. Fractions were collected manually, and sequencing was carried out with a gas phase instrument (Applied Biosystems 477A).

**RESULTS**

Isolation of cDNA Clones Encoding the FMO Expressed in Rabbit Lung—A cDNA library constructed with rabbit pulmonary mRNA was screened with polyclonal antibodies to the FMO expressed in rabbit lung. The specificity of the antibody used for screening is shown in Fig. 1. Out of 50,000 plaques screened, seven clones were detected and isolated, and the longest insert (pFMO-1) was sequenced by the strat-
pFMO-1 terminates in a sequence of 10 adenosine nucleotides, upstream. However, three putative polyadenylation signals at the 5' and 3' ends of six additional clones isolated from the 3' sequence of the insert (see Fig. 2). The pFMO-1 alignment is discontinued in the 3'. The complete nucleotide sequence of pFMO-7 was also different in that it terminated in a sequence (CAAAA) that may represent part of a poly(A) tail for a transcript shorter than that of pFMO-1; two of the three potential poly(A) signals found 385 nucleotides upstream from the 3' terminus of pFMO-1 are located 16 and 22 bases from the 3' terminus of pFMO-7. No differences between the nucleotide sequences of pFMO-2 and 4, as well as that of pFMO-6, and pFMO-7 were identical to those of the liver and lung. Initiation and termination codons are marked. Nucleotide sequences of the flavin-containing monooxygenases expressed in rabbit liver and lung. The complete nucleotide sequence of pFMO-1 is reported in Fig. 3. Although pFMO-1 terminates in a sequence of 10 adenosine nucleotides, there is no consensus polyadenylation signal (AATAAA), or any of the occasionally seen variants (37), located 10–30 bases upstream. However, three putative polyadenylation signals (AATAAA) are found within a 36-base region beginning 385 bases from the 3' end of pFMO-1 (Fig. 2). The pFMO-1 sequences of four of these clones (pFMO-3, 5, 6, and 7) were identical to those of the corresponding regions of pFMO-1. In contrast, the 5' ends of both pFMO-2 and pFMO-4 differed from pFMO-1 at two positions; each contained a T in place of G at position 440 of pFMO-1 and a G in place of C at position 485 (Fig. 2). The 3' sequences of pFMO-2 and 4, as well as that of pFMO-6, also differed from that of pFMO-1 by the occurrence of a single deletion in a series of seven consecutive adenosines (bases 2109–2115) present in pFMO-1 (Fig. 2). The 3' sequences of pFMO-2 and 4 were also different in that they terminated in a sequence (CAAAA) that may represent part of a poly(A) tail for a transcript shorter than that of pFMO-1; two of the three potential poly(A) signals found 385 nucleotides upstream from the 3' end of pFMO-1 were located 16 and 22 bases from the 3' terminus of pFMO-7. No differences be-
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Isolation of cDNA Clones Encoding the FMO Expressed in Rabbit Liver—The cDNA library constructed with mRNA isolated from rabbit liver was screened with cDNA encoding the FMO expressed in pig liver (17). Six clones were detected, and one insert (hFMO-1) was sequenced by the strategy shown in Fig. 4. The complete sequence of hFMO-1 contains 2046 bases with an open reading frame of 1805 bases, a 5'-flanking region of 48 bases, and a 3'-flanking region of 390 bases. The sequence of hFMO-1, aligned with that of pFMO-1, is shown in Fig. 3.

Amino Acid Sequences Derived for the Forms of FMO Expressed in Rabbit Liver and Lung—The primary sequences derived for the FMO enzymes expressed in rabbit liver and lung are shown in Fig. 5, along with the sequence of the FMO expressed in pig liver (17). Both rabbit proteins contain 535 amino acids, compared with 532 for the pig enzyme. The calculated molecular mass of the liver FMO is 59,841 daltons and of the lung FMO is 61,145 daltons. The base substitutions seen with pulmonary pFMO-2 and pFMO-4, relative to the sequence of pFMO-1, form codons for different amino acids: serine for alanine at position 120 and glutamic acid for glutamic acid at position 136.

Fig. 4. Strategy used for sequencing cDNA encoding the flavin-containing monoxygenase expressed in rabbit liver. A graphical representation of clone hFMO-1 is shown at the top of the figure along with the fragments that were subcloned and sequenced. Fragments were obtained by restriction (arrows) or sonication (**). The restriction enzymes used were EcoRI (E), RsaI (R), A calf (A), and Sau3A (S).

Fig. 5. Comparison of the primary sequences of the flavin-containing monoxygenases expressed in rabbit liver and lung and pig liver. The complete amino acid sequence of the rabbit liver FMO is shown. Positions in the rabbit lung or pig liver sequences that contain the same amino acids as found in the rabbit liver sequence are indicated by an equi sign. Positions in the pig liver sequence that contain the same amino acids present in the rabbit lung sequence but not found in the rabbit liver sequence are indicated by a plus sign. Peptide sequences obtained from purified rabbit lung FMO are underlined. Identical sequences of 5 or more amino acids present in all three proteins are indicated by asterisks.

The two liver enzymes are much more similar (87% identity) and share several large areas of absolute identity, including the first 69 N-terminal amino acids and a 52-amino acid peptide beginning at position 328 of the rabbit liver sequence. Two 14-amino acid peptides, beginning at positions 166 and 328 of the rabbit liver sequence, are common to all three proteins. Alignment of the three amino acid sequences also reveals that the pig liver and rabbit lung enzymes share a 3-residue gap corresponding to positions 317, 318, and 319 of the rabbit liver sequence (Fig. 5).

Putative FAD and NADP pyrophosphate-binding domains are located at the same positions in the primary sequences of all three FMO enzymes (Table I). The FAD pyrophosphate-binding site contains a highly conserved consensus sequence of GxGxxG beginning at residue 9, and the NADP site contains a similar sequence (GxGxxG/A) beginning at residue 191. Similar binding domains, typically separated by about 175 residues, are present in a number of other enzymes, some of which (38-41) are included in Table I.

The hydropathy indices (42) of the proteins are shown in Fig. 6. As expected from the high identity of their primary sequences, the profiles of the rabbit liver and pig liver proteins are nearly superimposable. Somewhat unexpected, however, is the marked similarity between the profile of the rabbit lung FMO and that of the liver enzymes. In fact, the only notable differences between the lung and liver profiles are a hydrophilic peptide (centered at residue 418) and a hydrophobic peptide (centered at residue 461) present in the liver but not in the lung proteins. Prediction of membrane associated peptides by several methods (43-45) yields virtually identical results with the sequences of the liver FMOs and very similar results with the sequences of the liver and lung enzymes (Fig. 6).

Peptide Sequences Obtained from Purified Rabbit Lung FMO—Because attempts to sequence intact rabbit lung FMO were unsuccessful (due likely to a blocked N terminus), the purified protein was digested with trypsin and the peptides separated by reverse-phase HPLC (Fig. 7). Four peptides (labeled 7, 17, 25, and 45) were selected for sequencing by an automated Edman’s procedure. The sequence of each peptide was found to be identical to a sequence present in the primary structure of the protein derived from the pulmonary cDNA clone (pFMO-1), and each sequence was flanked by an appropriate cleavage residue (Fig. 5).

Analysis of mRNA from Rabbit Liver, Lung, and Kidney and Genomic DNA from Liver—Samples of hepatic, pulmonary,
and renal mRNA were separated by electrophoresis in agarose gels (1%) containing methyl mercury (0.5%), transferred to nylon membranes, and hybridized with cDNA encoding hepatic (hFMO-1) and pulmonary (pFMO-1) FMO. Single bands of mRNA (2.6 kb) were detected in the hepatic and renal samples by hybridization with hFMO-1; no pulmonary mRNA was detected with this probe (Fig. 8). In contrast, no hepatic mRNA was detected with pFMO-1, whereas four distinct bands of mRNA (9.4, 2.6, 4.8, and 6.0 kb) were detected in the pulmonary and renal samples (Fig. 8). The relative intensities of these four bands were the same in 12 pulmonary mRNA samples prepared from individual rabbits (not shown). The same relative intensities were also obtained at different hybridization temperatures (42, 52, and 65°C) and with 5' (bases 1–521) and 3' (bases 1368–2611) probes prepared from pFMO-1 by restriction with PstI (not shown).

The 3' and 5' pulmonary pFMO-1 probes described above and 5' (bases 1–735) and 3' (bases 736–2046) hepatic probes, prepared by restriction of hFMO-1 with KpnI, along with full-length pFMO-1 and hFMO-1 were used to analyze genomic DNA isolated from liver (Fig. 9, a and b). The DNA was restricted by incubation with Ncol, HindIII, EcoRI, or PstI, and the fragments were separated electrophoretically in agarose gels (0.7%) and transferred to nylon membranes. Hybridization to the 3' probes provided the least complicated results in that single bands were observed in all but one case. The exception was that two bands were obtained when DNA restricted with EcoRI was hybridized to the 3' hepatic probe (Fig. 9b). The larger (~9.0 kb) of these fragments was also detected by the 5' hepatic probe and appeared to be due to cross-hybridization with the 3' end of the gene encoding the pulmonary enzyme (Fig. 9a).

The 5' pulmonary probe hybridized with two (Ncol and HindIII) or three (EcoRI and PstI) DNA fragments, some of which were also detected with the 3' probe. The full-length pulmonary pFMO-1 hybridized to the same fragments as the 3' and 5' probes, as well as with several others assumed to represent the area of the gene associated with bases 522–1367 of pFMO-1. With Ncol, for example, fragments of 3.0 (5'), 4.0 (3'), 4.4 (internal), and 5.0 kb (5') were detected. Two internal fragments were detected with HindIII and two with PstI. As expected, the full-length hepatic hFMO-1, did not detect any fragments in addition to those detected by the 3' and 5' hepatic hFMO-1 probes.

**DISCUSSION**

Consideration of the substrate specificities, immunochemical properties, and certain physical parameters of the FMO expressed in liver and lung suggests that these enzymes might be more analogous than homologous. Their catalytic activities do differ (12–16) but remarkably little in view of the disparities between their immunochemical properties (13, 14, 46), stabilities (13), and responses to Hg2+ (11). As a first step in ascertaining the structural bases for the similar overall catalytic activities, specific substrate differences, and distinct physical properties of these enzymes, we have derived their primary structures from the nucleotide sequences of cloned cDNAs. Several structural characteristics of the FMOs expressed in rabbit liver and lung in pig liver are compared in Fig. 10.

The amino acid sequences derived for the FMO enzymes expressed in lung and liver are 56% identical, a clear indication of homology. However, the extent of the differences between these primary structures is indicative of an evolutionary divergence that occurred prior to speciation. This is apparent when the much higher identity (87%) between the FMO enzymes expressed in livers of different species (rabbit and pig) is considered (Fig. 10). In this respect, it is noteworthy that the sequences of the rabbit lung and pig liver proteins (57% identity) have certain similarities not shared with the

**Table I**

| Enzyme | FAD-binding domain | NADPH-binding domain |
|--------|---------------------|-----------------------|
|        | Amino acid no. | Sequence | Amino acid no. | Sequence |
| FMO, liver | 4 | KVAVIGAGVSGLISLKL | 186 | RVLVGGNSGDIAV |
| FMO, lung | 4 | KVAVIGAGVSGLISK | 186 | RILVIGNSASDIAV |
| TMO | 40 | KVAVIGAGSVGHVAT | 233 | LPGSHGSGGLYPY |
| MRAse | 100 | KVAVIGAGSAAMAL | 272 | LVAVGSVRAELAQ |
| ADR | 8 | QTVVGAGSTGTPITAA | 148 | TAVTIQGNNVAVNHR |
| GRase | 22 | DLYVGGAGGGLASAR | 189 | RSVVAGYIAYEMAG |

* Flavin-containing monoxygenase expressed in rabbit and pig liver.
* Flavin-containing monoxygenase expressed in rabbit lung.
* Tryptophan 2-hydrolase expressed in Pseudomonas aeruginosa (38).
* Mercapturic reductase expressed in Pseudomonas aeruginosa (39).
* Adrenodoxin reductase expressed in bovine adrenal cortex (40).
* Glutathione reductase expressed in human erythrocytes (41).
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FIG. 7. HPLC chromatogram of peptides generated by digestion of purified rabbit pulmonary FMO with trypsin. The peptides were separated on a C-18 column and their elution monitored at 214 nm. Peptides labeled 7, 17, 25, and 45 were sequenced by an automated Edman's procedure.

FIG. 8. Hybridization of cDNAs encoding the FMO expressed in rabbit liver and lung to mRNA prepared from rabbit tissues. Messenger RNA was electrophoresed in an agarose gel, transferred to a nylon membrane, and hybridized to cDNAs labeled by the random priming method. Liver, hFMO-1 cDNA was hybridized to hepatic (lane 1, 2 μg), renal (lane 2, 5 μg), and pulmonary (lane 3, 10 μg) mRNA. Lung, pFMO-1 cDNA was hybridized to pulmonary (lane 1, 2 μg), renal (lane 2, 5 μg), and hepatic (lane 3, 10 μg) mRNA.

rabbit liver enzyme. Notwithstanding the marked differences between their sequences, the structures of the FMO enzymes expressed in liver and lung have a number of common features: their pyrophosphate-binding domains are nearly identical, and they have five putative membrane-associated regions in common, including two in areas that have little primary sequence identity (Fig. 10). These common properties indicate that the overall structural similarity between the pulmonary and hepatic enzymes is likely greater than might be predicted from the percent identity of their primary sequences.

The marked differences between the primary structures of the FMO proteins expressed in lung and liver are sufficient evidence for concluding that these enzymes are products of distinct genes, a conclusion confirmed by analysis of genomic DNA. Although several restriction enzymes yield DNA fragments associated with both enzymes, these are readily explained by cross-hybridization of the probes with genomic DNA containing one or more of the eight identical nucleotide sequences (11-21 bases in length) present in the coding regions of the lung and liver sequences. The fragmentation patterns observed with genomic DNA indicate that the pulmonary and hepatic enzymes may not be encoded for by more than one gene each. In any case, hybridization of genomic DNA with lung or liver cDNA presents the same general picture and offers no explanation for why the pulmonary, but not the hepatic, enzyme is associated with multiple populations of mRNA.

Initially, detection of multiple bands of pulmonary mRNA upon hybridization with pFMO-1 was thought to be related in some way to the multiple bands of pulmonary microsomal
protein detected with antibodies to the FMO expressed in lung (40). This idea was reinforced by detection of the same mRNA and protein bands in samples from kidney (this work and 46). However, in contrast to expression of the protein bands in three distinct phenotypes (46), identical mRNA bands were present in pulmonary samples from 12 individuals examined. Also, results of preliminary studies reveal that pFMO-1 hybridizes to a single band of pulmonary mRNA from guinea pig (not shown), a second species for which multiple protein bands are associated with the FMO expressed in lung (46). Based on studies with 3' and 5' probes and analyses carried out at different temperatures, cross-hybridization of pFMO-1 with similar but distinctly different mRNAs was also ruled out as an explanation for the multiple bands seen with pulmonary and renal samples. These bands are likely derived from the same transcript by alternative splicing or variable 3' processing, the latter being consistent with the presence of multiple putative polyadenylation sites (47). The largest band, probably an unusually stable or only partially processed transcript, is not represented in our cDNA library because of the size restrictions we used. On the other hand, the smallest band (about 2.4 kb) is likely represented by clone pFMO-7, which terminates in CAAA, a sequence that is not present in the other clones and occurs 16 bases downstream from a polyadenylation signal.

The existence of allelic variants of the FMO expressed in lung may explain, at least in part, the multiple protein bands observed. Data from sequencing show at least two populations of cDNAs which vary by less than 1% in the regions examined. However, the two base differences detected in the coding regions of these cDNAs do result in amino acid changes. Such variability between proteins having the same number of residues has little effect on monomeric molecular weight but can alter mobility in polyacrylamide gels in the presence of SDS, as has been observed with rat cytochrome P-450 isozymes b and e (48). The contribution of allelic variants and the potential involvement of post-translational modification to formation of the multiple proteins bands associated with the FMO expressed in lung are being investigated further.

In conclusion, we have derived sequences for the FMO enzymes expressed in rabbit liver and lung. The relationship between these proteins suggests an evolutionary divergence that took place prior to speciation. The lung, but not the liver, FMO is associated with multiple species of mRNA. These mRNAs do not appear to be related to the multiple protein bands detected with antibodies to the FMO expressed in lung; however, their exact nature is not yet understood. In the next stage of our work, we will attempt to define the structural elements responsible for differences between the catalytic and physical properties of the FMOs expressed in lung and liver.

Note Added in Proof—While this manuscript was in preparation, evidence for two related forms of flavin-containing monooxygenase in rabbit liver was reported by Ozols (Ozols, J. (1989) Biochem. Biophys. Res. Commun. 163, 49-55). Sequence data obtained from two purified proteins were given for two regions, one including or near the amino terminus and one internal region of about 40 residues. The peptides from one protein (form 1) are nearly identical with peptides found in the sequence we report for the hepatic FMO. On the other hand, the sequences from the second protein (form 2), which are 80% (amino-terminal) and 60% (internal peptide) identical with those from form 1, do not match with sequences from the pulmonary FMO. Thus, it appears that the flavin-containing monooxygenase may consist of at least three related but distinct gene products.
The flavin-containing monooxygenase enzymes expressed in rabbit liver and lung are products of related but distinctly different genes.
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