SPECIES, ORGAN AND CELLULAR VARIATION IN THE FLAVIN-CONTAINING MONOOXYGENASE

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CONTENTS

SUMMARY

The distribution of the flavin-containing monooxygenase (EC1.14.13.8) (FMO) between species, organs and cell types is summarized with particular reference to the organ specific forms present in mammalian lung and liver. The role of the FMO relative to cytochrome P-450 in the oxidation of the sulfur atoms of organosulfur compounds is considered with particular reference to the hepatatoxicant thiobenzamide, the insecticide phorate and the drug, thioridazine. Of special interest is the relative role of these enzymes in complex metabolic pathways of xenobiotics.

I. INTRODUCTION

The flavin-containing monooxygenase (EC 1.14.13.8) (FMO), originally described as an amine oxidase, was subsequently shown to be a versatile sulfur oxidase. These early studies were summarized by Ziegler /1/. More recently the FMO has been shown to be a phosphorus oxidase /2,3/. This enzyme and the cytochrome P-450-dependent monooxygenase system are the principal enzymes catalyzing the oxidation of lipophilic xenobiotics to electrophilic products capable of further metabolism, often to readily excretable conjugation products. Both enzymes are microsomal and require NADPH and molecular oxygen for activity.

A great deal is known about the substrate specificity of the FMO, much of it summarized in a recent review /4/. Purification of pig liver FMO was accomplished some time ago /5/ and the capability of the solubilized enzyme to catalyze the oxidation of the same wide variety of nucleophilic nitrogen, sulfur and phosporus compounds as the membrane-bound enzyme was established /1-5/. The physiological substrate for this enzyme is thought to be cysteamine, which is oxidized to cystamine, providing a thiol oxidant for the synthesis of peptide disulfides /6/.

More recently, it has been demonstrated that the FMO exists in many species and in more than one organ. Furthermore, the FMO has been purified from a number of sources. It appears appropriate to explore, in the light of these new findings, the distribution and role of the FMO, particularly as they relate to the oxidation of organic sulfur compounds.

This mini-review was developed with relatively little change from a communication presented at the First International Symposium on Sulfur Xenobiochemistry under the title "S-Oxygenases: Substrate Specificities of Various Flavin-Containing Monooxygenases". Space limitations preclude an exhaustive review and, in the spirit of the original presentation, emphasis is placed on contributions from the authors laboratory.

II. SPECIES DIFFERENCES

Although it has been known for some time that the FMO exists in more than one species /1/ it is only recently that detailed comparisons have been made between the FMO activity in microsomes from the same organ of different species. Similarly, detailed comparisons of purified FMOs from different species are now possible because of the development of newer purification methods /7/. The first comprehensive comparison of species and organs /8/ employed an immunochemical method. Although this study provided useful qualitative information, the quantitative estimates, particularly for extrahepatic tissues, are almost certainly low due to lack of knowledge, at that time, of the immunologically different forms of the enzyme.

A comparison of the substrate specificity of liver FMOs of several species is shown in Table 1 and a comparison of the purified enzymes from mouse and pig liver is shown in Table 2. These enzymes are generally similar with some relatively minor differences. Lung FMOs will be discussed in more detail later but it may be noted that the lung FMOs from different species are broadly similar to each other but differ from the hepatic enzyme of the same or different species.

One or more forms of FMO have been characterized in microsomes from pig, mouse, rat, rabbit, guinea pig, hamster, dog, human and *Trypanosoma cruzi* and one or more forms of the enzyme have been purified from pig, mouse, rat, rabbit, human and *Trypanosoma cruzi* /1,4,7-15/.

TABLE 1

Species Variation in the Oxidation of
Sulfur-containing Compounds by the FMO of Hepatic Microsomes

Substrate	Activity[1] Mouse	Rat	Rabbit	Pig
Thiourea	18.9	7.8	12.3	12.9 (31.4)[2]
Phenylthiourea	9.0	4.4	7.7	9.3 (26.1)
α-Naphthylthiourea	7.3	4.0	6.6	7.9 (21.6)
Methimazole	17.4	7.2	10.2	14.3 (30.4)
2-Mercaptobenzimidazole	6.1	2.9	6.1	8.3 (18.8)
Ethylene sulfide	16.6	6.7	11.0	11.3 (21.7)
Methylphenylsulfide	10.2	4.7	7.1	9.9 (25.6)
Thioacetamide	18.6	7.5	11.6	13.4 (31.0)
Cysteamine	17.8	8.1	11.6	13.6 (30.9)
l-Butanethiol	9.2	4.1	7.2	7.8 (20.7)
Trans-o-Dithiane-4,5-diol	4.8	2.5	6.9	7.3 (10.9)

[1] Activity:nmol NADPH/min/mg microsomal protein.
[2] Values in parenthes is determined in presence of n-octylamine.
Modified from Tynes & Hodgson /11/.

III. ORGAN DIFFERENCES

Differences in catalytic activity between microsomes from different organs have often been determined and it is clear that the activity in lung and kidney may be as high as that of the liver. One such comparison is shown in Table 3.

The most extensive comparison of FMOs of different organs is between those of the liver and the lung. The first indication that these FMOs might differ from one another was provided by Devereux et al. /17/ who reported marked differences in the effects of Hg^{2+} on partially purified FMO preparations. Subsequently, differences in lung and liver FMOs were suggested by the studies of Ohmiya and Mehendale /18,19/ on chlorpromazine and imipramine metabolism in the lung of the rat and the rabbit. These and other compounds are substrates for the liver, but not the lung, FMO.

More recently, our group /20/ as well as Williams et al. /21,22/

TABLE 2

Comparison of FMO Purified from Mouse and Pig Liver

Characteristic	Mouse[1]	Pig[2]
Binding to Blue Agarose	+	+
Binding to Red Agarose	+	+
Binding to AMP Agarose	+	+
Binding to ADP Agarose	+	+
Molecular Weight	58,000[3]	56,000[3] 64,000[4] 56,000[3]
Spectral Maxima (oxidized)	383,453	382,450
pH Optimum	9.5	8.5
Km (M)(pH 7.6)		
Methimazole	10	5
NADPH	5	5
NADH	86	>50

1 Data from /9/
2 Literature values
3 By SDS-PAGE
4 By flavin analysis

purified an FMO from rabbit lung that was shown to be catalytically and immunologically distinct from the liver enzyme. The mouse and rabbit lung FMOs have a unique ability for N-oxidation of primary aliphatic amines, including n-octylamine, a positive effector but not a substrate for the liver enzyme and also a compound often added to microsomal incubations to inhibit cytochrome P-450 (P-450) /20,23,24/. In the mouse lung n-octylamine is both a substrate and a positive effector. The mouse and rabbit lung enzymes have a higher pH optimum, near 9.8, compared to that of the FMO from the liver which is approximately 8.8. Using antibodies raised in goats, Ouchterlony immunodiffusion analysis showed that the liver and lung proteins were immunochemically dissimilar /20/. It has now become evident that there are several FMO isozymes with overlapping

TABLE 3

Comparative Rates of Sulfur Metabolism in Selected Pesticide Substrates by the FMO of Mouse Liver, Lung and Kidney Microsomes[1]

Substrate	Activity (nmoles NADPH/min/mg) Liver	Lung	Kidney
Phorate	6.47	9.98	5.44
Disulfoton	8.58	12.31	8.23
Fenthlon	5.77	2.33	–[2]
Methyl Carbophenothon	3.76	2.86	–[2]
Croneton	3.31	2.00	–[2]
Aldicarb	1.65	1.13	1.79

[1] Data selected from /16/
[2] Not determined

substrate specificities, and it is likely that the relative proportions of these isozymes vary in different tissues within and between species /12/.

Although there have been no detailed studies of the FMOs from tissues other than liver and lung, apparent FMO activity has also been noted in kidney, bladder mucosa, testes, corpus luteum, thyroid, thymus, adrenal gland, placenta, aorta, lymph nodes, pancreas, small intestine and skin /1,8,25,26/.

IV. CELLULAR DIFFERENCES

Recently, studies of the cellular localization of the FMO have been carried out (Overly, L., Lawton, M., Philpot R.M. and Hodgson, E., unpublished results). An immunohistochemical method utilizing peroxidase labelled antibodies and diaminobenzidine revealed that in the rabbit lung the FMO is highly localized in the non-ciliated bronchiolar epithelial (Clara) cells. The lung FMO did not cross-react with the antibody to the liver enzyme.

V. RELATIVE IMPORTANCE OF THE FMO IN MICROSOMAL OXIDATIONS IN DIFFERENT TISSUES

The same substrate may be oxidized by both P-450 and the FMO; this is especially prevalent with sulfur containing pesticides and drugs. Although a small number of studies /27, 28/ have demonstrated the oxidation of N,N-dimethylaniline by both P-450 and the FMO in microsomes from the same organ, the relative contributions of the two enzymes were not closely quantitated. Recently, in order to study the relative contributions of these two enzymes with common substrates, methods were developed to measure each separately in the same microsomal preparation. Two techniques have been particularly useful. The first involves the inhibition of P-450 activity by the use of an antibody to NADPH cytochrome P-450 reductase, thus permitting the measurement of FMO activity alone. The second consists of heat treatment of the microsomal preparation (50C for 1 min.) to inactivate the FMO, thus permitting measurement of P-450 alone, as P-450 activity is unchanged by the heat treatment /29,30/.

TABLE 4

Relative Contributions of FMO and P-450 to the Microsomal Oxidation of Thiobenzamide in Microsomes from Rat and Mouse Tissues

Species	Sex	Tissues	Relative Activity (%) P-450	FMO
Mouse	M	Liver	50	50
Mouse-Pb[1]	M	Liver	65	35
Mouse	F	Liver	25	75
Mouse	M	Lung	20	80
Rat	M	Liver	35	65
Rat	M	Lung	40	60

[1] Mice pretreated with phenobarbital

Modified from Tynes and Hodgson 1983, /29/

The relative contributions of the two enzyme systems with thiobenzamide as substrate are summarized in Table 4. Note the higher FMO contribution in female than in male mouse liver as well as the difference in relative contribution between liver and lung in the mouse but not in the rat. Similar studies have been carried out using the insecticide, phorate, examining the relative contribution of the two systems in the production of phorate sulfoxide in different tissues (Table 5) /31 and Kinsler, Levi and Hodgson, unpublished data/. In the livers of untreated animals P-450 is more important in the sulfoxidation of phorate (P-450:FMO, approximately 75:25); by contrast in the kidney and lung, while the overall activity is low compared to the liver, the relative contribution by the FMO is significantly higher. This is particularly evident in microsomes from female mice in which 90 percent of phorate sulfoxidation is due to FMO.

VI. FACTORS AFFECTING RELATIVE IMPORTANCE

Relative levels of activity are easily disturbed by compounds or conditions that alter either the level of P-450 or the level of the FMO. Thus pretreatment of mice with phenobarbital significantly increases, not only the overall rate of phorate oxidation, but also the relative contribution of the P-450 pathway (Table 5).

Piperonyl butoxide is a methylenedioxyphenyl compound with a biphasic effect on P-450 activity in the liver, first inhibition and, subsequently, induction. In mice treated with piperonyl butoxide it can be demonstrated (Table 5) (Kinsler, Levi and Hodgson, unpublished data) that the proportion of phorate oxidation due to the FMO first rises, during the P-450 inhibition phase, and then falls, during the P-450 induction phase.

The effects of xenobiotics on the relative contributions of the FMO and P-450 appear to be mediated primarily via the P-450 component since the FMO does not appear to be inducible by xenobiotics. FMO levels may, however, vary with nutrition, diurnal rhythms, sex, pregnancy and corticosteroids, such as dihydrocortisone, although the effects appear to be both species and tissue dependent (see 1,4 for references).

We have also studied (Kinsler, Levi and Hodgson, unpublished results) the effects of hydrocortisone treatment on the metabolism of

TABLE 5

Relative Contributions of P-450 and FMO to the Microsomal Oxidation of Phorate in Microsomes from Several Mouse Tissues

Tissue	Sex	Product Formation (nmoles phorate sulfoxide/mg protein/min) Control	+ARa	+Heat	%FMO	%P-450
Liver	M	12.7	2.8	9.3	21.7	78.3
Liver	F	14.4	3.7	11.7	24.0	76.1
Lung	M	3.3	1.9	–	59.1	41.3
Lung	F	5.7	3.1	–	54.0	46.0
Kidney	M	1.6	1.2	–	72.0	28.1
Kidney	F	2.0	1.8	–	90.0	10.0
Liver-PB[2]	M	69.7	10.1	59.6	14.3	85.5
Liver-PBO[3]						
2 hr	M	11.1	–	6.5	41.4	58.6
6 hr	M	19.4	16.3	–	16.0	84.0

1 Antibody to P-450 reductase

2 Phenobarbital treated mice

3 Piperonyl butoxide treated mice

Data from /31/ & Kinsler, Levi and Hodgson unpublished data

phorate and thiobenzamide by the FMO of mouse liver and lung. As shown in table 6, the FMO activity in the liver is increased for both substrates (+82% for phorate, +52% for thiobenzamide) with only minor changes in the lung (-15% for phorate, +20% for thiobenzamide).

Such alterations may assume toxicological importance when the products from the two enzymes differ, particularly when one metabolite is more toxic or more pharmacologically active than others.

TABLE 6

FMO Oxidation of Phorate and Thiobenzamide by Liver and Lung Microsomes from Hydrocortisone Treated Female Mice

		FMO Activity (nmol product/min/mg protein)	
		Phorate	Thiobenzamide
Liver			
	Control	1.7	3.62
	Treated	3.1	5.55
Lung			
	Control	4.6	2.17
	Treated	4.0	2.61

From Kinsler, Levi and Hodgson unpublished data.

VII. CONTRIBUTION OF THE FMO TO COMPLEX METABOLITE PATHWAYS

One of the most interesting and relatively unexplored aspects of FMO function is its contribution to complex metabolic pathways of xenobiotics. It is clear that both purified enzymes and intact microsomes, using the methods described above, must be used to elucidate the reactions and products involved in complex metabolic pathways, especially when several enzymes are involved and the oxidative pathways involve both detoxication and activation reactions.

Recently we utilized purified FMO and P-450 isozymes to examine in detail the oxidative pathways of phorate metabolism /32,33/.

Both P-450 and FMO catalyze the initial sulfoxidation of thioether-containing organophosphate insecticides such as phorate and disulfoton to form the sulfoxide. Subsequent oxidation reactions, however, such as formation of the sulfone and oxidative desulfuration to the corresponding oxons are catalyzed entirely by P-450. Although both the FMO and P-450 catalyze the initial sulfoxidation, the products are stereochemically different. The FMO forms the (−) phorate sulfoxide while two of the P-450 isozymes (P-450 B2, a major constitutive form, and P-450 PB, the principal form induced by phenobarbital) yield (+) phorate sulfoxide. The other three P-450 isozymes examined gave racemic mixtures.

Both (+) and (−) phorate sulfoxide are substrates for further oxidation by P-450 to either the oxon (an activation reaction) or the sulfone (a detoxication pathway). However, not only is (+) phorate sulfoxide the preferred substrate, but the percent of oxon sulfoxide relative to the percent sulfone is higher with the (+) sulfoxide as substrate. It is interesting to note that the isozyme of P-450 induced by phenobarbital (P-450 PB) not only forms the (+) phorate sulfoxide more rapidly but also produces the highest percentage of oxon sulfoxide of any of the P-450s. Clearly environmental or physiological factors which increase the level of this isozyme in vivo could potentially enhance the toxicity of this compound.

Thioridazine is a phenothiazine neuroleptic that is extensively metabolized after administration. Examination of the chemical structure of thioridazine relative to known substrates for the FMO and P-450 isozymes indicate the likelihood that it is a substrate for both of these monooxygenases. S-oxidation is known to be the predominant route of metabolism in man /34,35/ producing the 2-sulfoxide, the 2-sulfone, and the 5-sulfoxide, the 2-sulfoxide and the 2-sulfone having greater antipsychotic activity than the parent compound /35,36/ while the ring sulfoxides appear to be largely responsible for the cardiotoxic side effects of thioridazine /37/.

Northioridazine, the demethylation product, is formed in significant quantities in rats, but not in man. Additional minor metabolites include ring hydroxylations and combinations of the above sulfoxides, sulfones, demethylation products, and phenols. Preliminary results (Lembke, Mailman and Hodgson, unpublished results) indicate the involvement of both P-450 and FMO in the oxidation of thioridazine.

Incubations with partially purified mouse FMO (P-450 free) and thioridazine in which NADPH consumption was monitored gave an Km of 8.8μM and Vmax of 352 nmol/min/unit FMO, indicating that thioridazine is, in fact, a good substrate for the FMO.

Heat pretreatment of microsomes caused a decrease in the amounts of the 2-sulfoxide and northioridazine (an N-demethylation reaction), indicating that these reactions may be mediated in part by the FMO. On the other hand the amount of the 5-sulfoxide and 5-sulfone produced is increased in incubations with heat treated microsomes. Such differential routes of metabolism may assume pharmacological significance in the case of activation of a prodrug or when one of the metabolites is more toxic.

VIII. CONCLUSIONS

The pioneering work of Zeigler and associates established that the FMO was a versatile nitrogen and sulfur oxidase. The enzyme was first purified from pig liver, the reaction mechanism was described, and the physiological role of the enzyme investigated. Recently, several laboratories including our own, have greatly extended the range of known sustrates as well as knowledge of the enzyme in several species and organs. Purification of the FMO from other species and organs has now established that immunochemically distinct forms of the enzyme exist and that these forms differ in physical properties and substrate specificity.

There are a number of exciting prospects for the immediate future. Investigation of the importance of the FMO relative to other monooxygenases, particularly in complex metabolic pathways for xenobiotic metabolism, has just begun. Further studies are critical for an understanding of the role of this enzyme in toxicological and pharmacological events. It is also clear that all of the background is now in place to permit systematic investigations of the molecular biology of the FMO isozymes.

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