Vol. 125, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1984 Pages 116-122

RABBIT LUNG FLAVIN-CONTAINING MONOOXYGENASE IS IMMUNOCHEMICALLY AND CATALYTICALLY DISTINCT FROM THE LIVER ENZYME[1]

D.E. WILLIAMS, D.M. ZIEGLER[2], D.J. NORDIN, S.E. HALE and B.S.S. MASTERS

Department of Biochemistry
Medical College of Wisconsin
Milwaukee, WI 53226

Received September 25, 1984

SUMMARY. Flavin-containing monooxygenase has been purified to homogeneity from lung microsomes of pregnant rabbits. Antibody to rabbit lung flavin-containing monooxygenase was raised in guinea pig. Ouchterlony double diffusion analysis with this antibody produced precipitin lines of identity with the purified rabbit lung enzyme and lung microsomes from pregnant and non-pregnant rabbits. No cross-reaction was seen with liver microsomes from rabbit or with purified pig liver enzyme.

The tricyclic antidepressant drugs, imipramine and chlorpromazine, are not substrates for the rabbit lung enzyme, whereas they are rapidly oxidized by the pig liver enzyme.

These results indicate that rabbit lung and liver flavin-containing monooxygenases differ in substrate specificity, are immunochemically distinct proteins, and may be different gene products. © 1984 Academic Press, Inc.

The flavin-containing monooxygenase (EC 1.14.13.8) purified to homogeneity from pig liver microsomes catalyzes oxygenation of a wide variety of drugs and other xenobiotics that possess a nucleophilic heteroatom (1,2,3). The oxygenation of alicyclic tertiary amines yields stable amine oxides and the NADPH- and oxygen-dependent oxidation of DMA[3] to DMA-N-oxide is frequently used to measure activity of this enzyme in mammalian tissues (4). While this activity can be detected in virtually all nucleated cells, most of the older studies on the characterization of the flavin-containing monooxygenase have been restricted to the pig liver enzyme. More recently, the enzyme has also been purified from rat (5), rabbit (6) and mouse (7) liver. Although some

[1]Supported by USPHS GM 31296 to BSSM.
[2]Department of Chemistry, Clayton Foundation Biochemical Institute, University of Texas at Austin, Austin, TX 78712
[3]Abbreviations used:
DMA, N,N-dimethylaniline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

0006-291X/84 $1.50
Copyright © 1984 by Academic Press, Inc.
All rights of reproduction in any form reserved.

species differences in kinetic constants for oxygenation of amine substrates have been observed (7), the liver enzyme from all species examined appears similar in composition, thermal stability, basic catalytic properties and substrate specificity.

On the other hand, a number of studies suggests that the DMA-N-oxidase of rabbit lung microsomes differs from liver microsomes in several respects. The activity in lung tissue increases during pregnancy and, in contrast to liver, is induced by pretreating rabbits with glucocorticoids (8). The DMA-N-oxidase of rabbit lung and liver microsomes also differs somewhat in optimal pH and in their response to Hg^{2+} (9-11). In addition, the extensive studies of Ohmiya and Mehendale (12-14) clearly show that rabbit lung microsomes do not catalyze N-oxygenation of imipramine and chlorpromazine - two of the better amine substrates for the liver flavin-containing monooxygenase.

We have recently purified flavin-containing monooxygenase from lung microsomes of pregnant rabbits (15) and found it to resemble the pig liver enzyme with respect to minimum molecular weight on SDS-PAGE, spectral properties, and activity with DMA, cysteamine and methimazole as substrates. However, the rabbit lung enzyme has a distinct pH profile, is stable in the presence of anionic detergents and to preincubation at elevated temperatures, and is not active towards benzphetamine nor stimulated by n-octylamine.

In this report, we demonstrate that the purified rabbit lung enzyme is immunochemically distinct from the rabbit and pig liver enzymes and that the amine substrate activity of the rabbit lung enzyme is much more restricted than that of the liver enzyme.

MATERIALS AND METHODS

The procedure for the purification of flavin-containing monooxygenase from pregnant rabbit lung microsomes will appear in detail elsewhere[4].

Briefly, pregnant (28th day of gestation) New Zealand white rabbits (3-5 kg) were killed by intracardiac injection of a saturated KCl solution and the lungs immediately excised and perfused with ice-cold saline. Microsomes were prepared by differential centrifugation and solubilized with sodium cholate (0.6%, w/v). Solubilized flavin-containing monooxygenase was purified by

[4] D.E. Williams, S.E. Hale and B.S.S. Masters (1984). Submitted.

sequential chromatography on n-octylamino-Sepharose 4B, DEAE-Sepharose and hydroxylapatite. Detergent was removed from the final preparation by adsorption to hydroxylapatite.

Antibody to rabbit lung flavin-containing monooxygenase was raised in guinea pig by injecting 50 μg i.m. of purified enzyme in Freund's complete adjuvant and boosting with 25 μg two weeks later using incomplete Freund's adjuvant.

The activity of purified rabbit lung or pig liver flavin-containing monooxygenase with various substrates was followed via NADPH oxidation at 340 nm or by oxygen consumption essentially as described previously (1). Microsomal activities were calculated from the substrate-dependent rate of oxygen consumption or rate of formation of DMA-N-oxide (16)

Protein was determined by the method of Lowry et al. (17) with human serum albumin as standard.

RESULTS

Immunochemical comparison of rabbit lung enzyme with rabbit and pig liver enzymes

Ouchterlony double diffusion analysis demonstrated that guinea pig anti-rabbit lung flavin-containing monooxygenase-IgG cross-reacts with microsomes from lungs of nonpregnant and pregnant female rabbits, forming lines of identity with the purified antigen (Fig 1). No precipitin lines formed with solubilized liver microsomes from rabbits or with purified pig liver flavin-containing monooxygenase. Similarly, anti-pig liver flavin-containing monooxygenase-IgG (raised in rabbit) failed to cross-react with rabbit lung microsomes or with the purified rabbit lung enzyme although antibodies to the pig liver enzyme do cross-react with an antigen in rabbit liver microsomes (data not shown).

Substrate specificities of rabbit lung and pig liver flavin-containing monooxygenase

We previously reported (15) that methimazole, DMA and cysteamine were substrates for both the purified rabbit lung and pig liver flavin-containing monooxygenases. The activities of purified rabbit lung and liver flavin-containing monooxygenases towards cysteamine, as measured by NADPH oxidation or oxygen consumption (Table I), are equivalent (697-758 nmol min^{-1} mg^{-1}). These values, assuming a minimum molecular weight of 59,000, would correspond to turnover numbers of 41-45 min^{-1}. It is not possible to compare this turnover to the microsomal activity (20 nmol min^{-1} mg^{-1}) directly

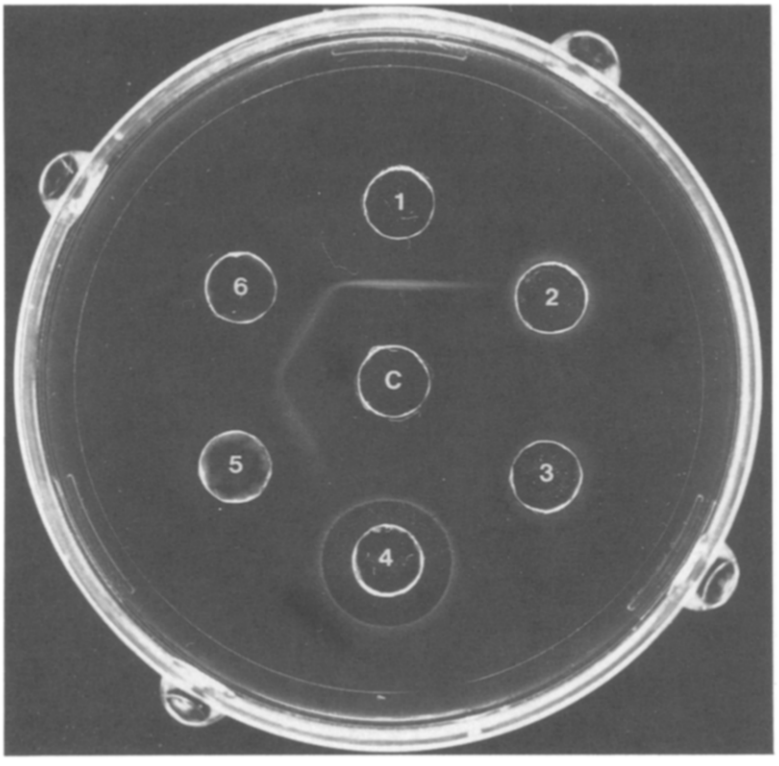

Fig. 1. Ouchterlony double diffusion analysis of rabbit and pig flavin-containing monooxygenase. Well C, 1 mg guinea pig antisera to rabbit lung flavin-containing monooxygenase; Well 1, purified rabbit lung enzyme (20 µg); Well 2, purified pig liver enzyme (20 µg); Wells 3 and 4, liver microsomes (0.25 mg) from pregnant and nonpregnant rabbits, respectively; wells 5 and 6, lung microsomes (0.25 mg) from nonpregnant and pregnant rabbits, respectively. Microsomes were solubilized for 30 min at 20°C with 1% Lubrol PX.

without a measurement of the microsomal specific content. Such a value would have to be obtained immunochemically. However, assuming that all the rabbit lung microsomal activity can be attributed to this purified enzyme, rabbit lung flavin-containing monooxygenase represents almost 3% of the total microsomal protein. This value is only slightly less than the concentration of flavin-containing monooxygenase in pig liver microsomes determined by an immunochemical method (18).

Imipramine and chlorpromazine are tertiary amines which have wide clinical applications as antidepressants, and are readily N-oxidized by pig liver flavin-containing monooxygenase (Table I). However, as we reported previously for benzphetamine, the purified rabbit lung enzyme is inactive

Table I. Substrate specificity of microsomal and purified rabbit lung flavin-containing monooxygenase (FMO)

	Specific Activity (nmol min^{-1} mg^{-1})			
	Rabbit Lung			Pig Liver
Substrate	microsomes[1]	FMO[1]	FMO[2]	FMO[2]
N,N-Dimethylaniline	11.3	-	922	729
Cysteamine	20.1	697	758	702
Methimazole	20.9	293	573	307
Imipramine	ND	ND	ND	367
Chlorpromazine	ND	ND	ND	502

[1]Activity was determined as nmol O_2 min^{-1} mg^{-1} using an oxygen electrode.

[2]Activity was determined as nmol NADPH oxidized min^{-1} mg^{-1} (340nm) using a UV/VIS Spectrophotometer.

ND = Not Detectable

towards these compounds when assayed by NADPH oxidation (Table I) or oxygen consumption (Table I and Fig. 2). This absence of activity was confirmed with rabbit lung microsomes. In the presence of SKF-525A (to inhibit any

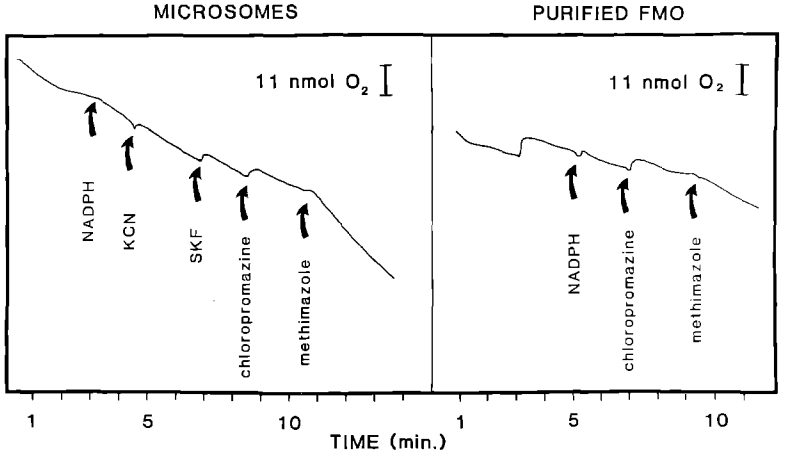

Fig. 2. Substrate-dependent oxygen consumption by pregnant rabbit lung microsomes and purified rabbit lung flavin-containing monooxygenase. The components were added to 0.6 ml of 0.1 M Tricine, pH 8.4, containing 0.5 mg of lung microsomal protein (left panel) or 15 µg of purified rabbit lung flavin-containing monooxygenase (right panel) at the time points indicated by the arrows. For determination of the microsomal activity, KCN was added to inhibit any activity due to mitochondrial contamination and SKF-525A to inhibit any oxygen consumption catalyzed by lung microsomal cytochrome P-450.

cytochrome P-450-dependent oxygen consumption), the addition of chlorpromazine (or imipramine) produces no increase in oxygen consumption over background (Fig. 2). This lack of turnover is not due to any inhibition of the enzyme as subsequent addition of methimazole produces a rate of oxygen consumption equivalent to that obtained in the absence of chlorpromazine (or imipramine).

DISCUSSION

Previous reports have suggested that rabbit lung flavin-containing monooxygenase is catalytically distinct from the rabbit or pig liver enzymes or the rat lung enzyme (9-14). A direct comparison has not been possible due to lack of the purified rabbit lung enzyme. We have purified flavin-containing monooxygenase from pregnant rabbit lung microsomes and utilized antibody, raised in guinea pig, to demonstrate that the lung enzyme is immunochemically distinct from the rabbit or pig liver enzymes. As is the case with rabbit antibody directed against the pig liver enzyme, the guinea pig-IgG did not inhibit DMA-N-oxidation by the purified rabbit lung enzyme (data not shown). Preliminary results with Western Blots of SDS-PAGE slab gels confirm the absence of any protein in rabbit liver microsomes corresponding to the rabbit lung enzyme.

The values listed in Table I confirm earlier reports (12-14) that rabbit lung microsomes do not catalyze the N-oxygenation of chlorpromazine or imipramine. This lack of activity can be explained by our finding that rabbit lung contains an immunochemically distinct flavin-containing monooxygenase that differs in substrate specificity from the liver enzyme. A similar enzyme may exist in goat or guinea pig lung, as lung microsomes from these species also display little or no activity towards imipramine and chlorpromazine (19).

Although mammalian liver flavin-containing monooxygenase is not inducible by administration of foreign compounds, the activity of this enzyme in mouse liver is regulated by sex steroids (20,21). The activity of the rabbit lung enzyme may also be under hormonal control since it is induced by glucocorticoids and is reported to increase almost 2-fold during late

gestation in pregnant rabbits (8). We have repeated these experiments and found the activity of the rabbit lung enzyme to increase four- to five-fold between the 25-28th day of gestation. The lung from pregnant rabbit should provide an excellent model for examining the induction of flavin-containing monooxygenase as well as providing another form of enzyme for studying the physiological function of mammalian flavin-containing monooxygenase.

REFERENCES

1. Ziegler, D.M. and Poulsen, L.L. (1978). Methods Enzymol, 52, 142-151.
2. Poulsen, L.L. and Ziegler, D.M. (1977). Arch. Biochem. Biophys. 183, 563-570.
3. Ziegler, D.M. and Poulsen, L.L. (1977). Trends in Biochem. Sci. 2, 79-81.
4. Ziegler, D.M., Duffel, M.W. and Poulsen, L.L. (1980). Sulfur in Biology pp. 191-204, Ciba Foundation Symposium 72.
5. Kimura, T., Kodama, M. and Nagata, C. (1983). Biochem. Biophys. Res. Commun. 110, 640-645.
6. Hlavica, P. and Hulsmann, S. (1979). Biochem.J. 182, 109-116.
7. Sabourin, P.J., Smyser, B.P. and Hodgson, E. (1984). Int. J. Biochem. 16, 713-720.
8. Devereux, T.R. and Fouts, J.R. (1975). Drug Metab. Dispos. 3, 254-258.
9. Devereux, T.R. and Fouts, J.R. (1974). Chem.-Biol. Interactions 8, 91-105.
10. Devereux, T.R., Philpot, R.M. and Fouts, J.R. (1977). Chem.-Biol. Interactions 18, 277-287.
11. Ohmiya, Y. and Mehendale, H.M. (1984). Drug Metabol. Disp. 12, 376-378.
12. Ohmiya, Y. and Mehendale, H.M. (1981). Pharmacology 22, 172-182.
13. Ohmiya, Y. and Mehendale, H.M. (1982). Biochem. Pharmacol. 31, 157-162.
14. Ohmiya, Y. and Mehendale, H.M. (1983). Biochem. Pharmacol. 32, 1281-1285.
15. Masters, B.S.S., Hale, S.E., Bedore, J.E. and Williams, D.E. (1984). Fed. Proc. 43, 2062.
16. Ziegler, D.M. and Pettit, F.H. (1964). Biochem. Biophys. Res. Commun. 15, 188-193.
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). J. Biol. Chem. 193, 265-275.
18. Dannan, G.A. and Guengerich, F.P. (1982). Molecular Pharmacol. 22, 787-794.
19. Ohmiya, Y. and Mehendale, H.M. (1984). Pharmacology 28, 289-295.
20. Wirth, P.J. and Thorgeirsson, S.S. (1978). Biochem. Pharmacol. 27, 601-603.
21. Duffel, M.W., Graham, J.M. and Ziegler, D.M. (1981). Molecular Pharmacol. 19, 134-139.