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Further Characterization of Rat Brain Flavin-Containing Monooxygenase

METABOLISM OF IMIPRAMINE TO ITS N-OXIDE

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ABSTRACT. Flavin-containing monooxygenase (FMO) activity was compared in rat liver and brain microsomes by estimating the actual amount of imipramine N-oxide relative to the corresponding activity, measured using substrate-stimulated rates of NADPH oxidation. The activities measured as NADPH oxidation rates were significantly higher than those estimated from the N-oxide formed. The brain FMO activity was detectable only in the presence of detergents (sodium cholate or Lubrol PX) or in microsomes that were freeze-thawed several times. The antibody to rabbit pulmonary FMO selectively inhibited imipramine N-oxidation. The antiserum to the rat liver NADPH cytochrome P-450 reductase had no effect on imipramine N-oxidation, indicating the noninvolvement of cytochrome P-450 in the above metabolic pathway. A flavin-containing monooxygenase was partially purified from the rat brain microsomes using sequential chromatography on *n*-octylamino-Sepharose 4B, DEAE-Sephacel and 2′,5′-ADP agarose. The purified FMO was resolved by SDS-PAGE into two bands (approximately 57 and 61 KDa, respectively) both of which cross-reacted with antibody to rabbit pulmonary FMO. The purified enzyme metabolized imipramine and the model substrate methimazole to their respective N-oxide and S-oxides. BIOCHEM PHARMACOL 51;11:1469–1475, 1996.

KEY WORDS. brain; antidepressants; flavin-containing monooxygenase; drug metabolism; monooxygenases; psychoactive drugs

FMO§ (EC 1.14.13.8) are mammalian flavoproteins involved in the oxidation of nitrogen- and sulphur-containing compounds. FMO activity has been detected in liver and certain extrahepatic organs, including lung and kidney [1–3]. Hepatic and pulmonary forms of FMO have been well-characterized and shown to be distinct proteins [4–7]. Recent studies from our laboratory have demonstrated the presence of FMO in the rat brain, and its ability to metabolize model substrates such as N, Ndimethylaniline, methimazole, and thiobenzamide [8] and the antidepressant drugs, imipramine, and fluoxetine [9]. Because several psychoactive agents are known to be substrates for FMO from other tissues, the local FMO-mediated metabolism of antidepressant drugs, such as imipramine, could have important modulatory effects on pharmacological activity (the N-oxide metabolites are inactive). Hence, the metabolism of imipramine to its N-oxide by rat brain FMO was investigated in some detail, by measuring the amount of N-oxide formed in comparison to the corresponding NADPH oxidation rates. The effects of detergents, pH, and temperature were also examined, and partial purification of the enzyme was undertaken.

MATERIALS AND METHODS

Sepharose 4B, 1,8-diaminooctane, DEAE-Sephacel, 2′,5′-ADP agarose, cholic acid, sodium cholate, Lubrol PX, Triton N-101, EDTA, Tris, NADPH, and *n*-octylamine were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A.. Other chemicals were purchased locally and were of analytical grade.

Wistar rats (3–4 months old), obtained from the Central Animal Research Facility of the National Institute of Mental Health and Neurosciences, Bangalore, India, were used for all experiments. Animals had free access to pelleted diet (Lipton India Ltd., Calcutta, India) and water. Prior to decapitation, animals were anaesthetized with ether and perfused transcardially with ice-cold buffer (0.1 M Tris, containing 1.15% KCl, pH 7.4). The brains were rapidly removed and the microsomes prepared and stored as described [9]. Microsomal protein content was determined by a dye-binding method [10] or by the method of Lowry *et al.* [11] using bovine serum albumin as standard. All buffers for

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§ *Abbreviations:* FMO, flavin-containing monooxygenase.

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FMO assay were oxygenated before use, unless stated otherwise.

FMO activity was assayed by measuring the substrate-stimulated rate of NADPH oxidation using methimazole, or imipramine as substrate. The assay buffer consisted of 0.1 M Tris HCl (pH 8.4) containing EDTA (1 mM), *n*-octylamine (3 mM) and Triton N-101 (0.1%, v/v). The assay was carried out essentially as described [9].

N-Oxidation of imipramine by rat brain microsomes was also determined by HPLC. The incubation mixture (1 mL) consisted of 0.2 M Tris HCl (pH 8.5), containing 1 mM EDTA, *n*-octylamine (3 mM), sodium cholate (0.6% w/v, unless stated otherwise), 5–40 μg of microsomal protein, and 1 mM NADPH. After preincubation for 3 min at 37°C, imipramine was added at a final concentration of 10 μM, unless indicated otherwise. After incubation at 37°C for varying periods of time (0.5–2 min), methanol (0.1 mL) was added to stop the reaction. The reaction mixture was mixed and centrifuged at 5000 rpm for 10 min. The supernatant was filtered through 0.22 μm nylon membrane and subjected to HPLC analyses. Blank incubations were carried out simultaneously without NADPH or using boiled microsomes. The HPLC analysis was carried out using a Supelco LC-CN column (15 cm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile: methanol: potassium phosphate buffer (0.01 M, pH 4.8) in the ratio of 25:15:60 (v/v). Metabolite identification was performed using a UV-detector set at 254 nm. Data acquisition from the HPLC output and peak integration were delayed by 5 min. Varying concentrations of standard imipramine N-oxide were also subjected to HPLC analyses and a standard curve was drawn using peak areas. The concentration of the N-oxide formed in the microsomal incubation was calculated from the standard curves. The minimum amount of imipramine N-oxide detectable by HPLC was 10 pmoles (3.33 ng).

The effect of the addition of various detergents, such as Triton N-101 (0.1%, v/v), Lubrol PX (0.1%, v/v), or sodium cholate (0.6%, w/v) on FMO-mediated metabolism of imipramine by rat brain microsomes was determined by adding any of these detergents, one at a time, to the assay buffer. The activity was also measured in the absence of detergents using microsomes that were freeze-thawed several times.

The buffer used for the activity measurements contained 0.2 M Tris and 1 mM EDTA and the pH of the buffer was adjusted variably from 6.5 to 10 with HCl, to study the effect of pH on FMO activity. The assay buffer also contained Triton N-101 (0.1%, v/v) and *n*-octylamine (3 mM). NADPH was added at a final concentration of 1 mM. FMO activity was measured using the assay buffers of varying pH (pH 6.5 to 10) by following the substrate-stimulated rate of NADPH oxidation at 340 nm, using imipramine as a substrate.

Microsomes were incubated at 45°C for 5 min, 10 min, 15 min, or 20 min. After the incubation, NADPH and substrate were added and the FMO activity was measured by estimating substrate-stimulated rates of NADPH oxidation.

Methimazole (3 mM) was added as a cosubstrate along with imipramine, after preincubation of the microsomes with NADPH, as detailed above. The FMO activity was assayed by measuring the N-oxide formed and by NADPH oxidation rates.

Rat brain microsomes (20 μg) were preincubated with antibody to rabbit pulmonary FMO (20 μL, 44 μg/μL) or antiserum to rat liver NADPH cytochrome P-450 reductase (20 μL) for 30 min at 4°C prior to the initiation of the assay by addition of NADPH and substrate. Microsomes preincubated with nonimmune goat IgG or nonimmune rabbit serum served as controls.

Purification of FMO

All the buffers used for purification were bubbled with nitrogen gas and contained 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 22 μM butylated hydroxytoluene. All the procedures were carried out at 4°C. The microsomes were thawed on ice and resuspended in 0.1 M potassium phosphate buffer (pH 7.25), containing 30% glycerol (v/v), to a protein concentration of 4 mg/mL and solubilized with sodium cholate (final concentration of 0.6%, w/v). The suspension was centrifuged at 100,000 *g* for 1 hr and the supernatant was collected. The supernatant was loaded onto a column packed with *n*-octylamino-Sepharose 4B that had been previously equilibrated with 0.1 M potassium phosphate buffer (pH 7.25) containing 20% glycerol (v/v) and 0.6% sodium cholate (w/v). The FMO was eluted with the above buffer containing 0.08% Lubrol PX (v/v). The fractions having flavin-containing monooxygenase activity were pooled, concentrated, and dialyzed. Following dialysis, the material was applied to a DEAE-Sephacel column that had been previously equilibrated with 10 mM potassium phosphate buffer (pH 7.7) containing 20% glycerol (v/v), 0.2% sodium cholate (w/v), and 0.1% Lubrol PX (v/v). The column was washed with the above buffer and the fractions containing FMO activity were eluted with the buffer containing 50 mM NaCl. All the fractions having FMO activity were pooled, concentrated, and loaded on a 2′,5′-ADP agarose column that had been previously equilibrated with 10 mM potassium phosphate buffer (pH 7.7) containing 20% glycerol (v/v) and 0.1% Lubrol PX (v/v). The column was washed with the same buffer and the FMO was eluted using a linear gradient of 0 to 0.1 mM NADPH. All the fractions having FMO activity were pooled and dialyzed.

The purified enzyme was subjected to SDS-polyacrylamide gel electrophoresis [12] and the gel was visualized using silver stain [13]. The molecular weight was estimated using standard molecular weight markers. Following electrophoresis, the protein was also transferred onto a nitrocellulose membrane for immunoblot analysis [14]. The immunoreactivity of the purified FMO was examined against antibody to rabbit pulmonary FMO. The metabolism of

imipramine to its N-oxide by purified brain FMO was also examined by HPLC, as given above.

RESULTS

The K_m and V_{max} for FMO-mediated N-oxidation of imipramine by rat liver and brain microsomes is depicted in Table 1. The affinity constants for FMO were determined by measuring the substrate-stimulated rate of NADPH oxidation and by measuring the amount of imipramine N-oxide formed by HPLC. The V_{max} for FMO-mediated oxidation of imipramine was higher in brain microsomes, regardless of the method of measurement (Table 1).

FMO activity could not be detected in freshly thawed brain microsomes in the absence of detergents using either of the two methods (Table 2). The activity was detectable only after addition of detergents such as sodium cholate, Lubrol PX, or Triton N-101 to the assay buffer. The formation of imipramine N-oxide could not be measured when Triton N-101 was added to the assay buffer, because this detergent interfered with the HPLC analysis. The FMO activity determined in the presence of detergents using the HPLC method (Fig. 1) was 43.1 and 44.4 nmoles of imipramine N-oxide formed/min/mg protein, which was similar to that observed in freeze-thawed (5 times) microsomes incubated in the absence of detergents (46.2 nmoles of imipramine N-oxide formed/min/mg protein). The enzyme activity was also estimated in detergent-containing buffer that was not bubbled with oxygen; the resulting activities were determined to be 37.9 nmoles of imipramine N-oxide formed/min/mg protein and 69.5 nmoles of NADPH oxidized/min/mg protein (data not shown). The FMO activity estimated as substrate-stimulated rates of NADPH oxidation varied between 69.7 to 77.5 nmoles of NADPH oxidized/min/mg protein in microsomes incubated in presence of nonionic detergents and in freeze-thawed microsomes. Maximal activity was, however, observed in microsomes incubated with sodium cholate (108.5 nmoles of NADPH oxidized/min/mg protein), which was significantly higher than that observed in the presence of other detergents (Table 2). In this instance also, the FMO activity measured as NADPH utilization rates was higher than the FMO activity measured as the amount of N-oxide formed. The FMO activity measured in the absence of *n*-octylamine was

TABLE 1. K_m and V_{max} of rat hepatic and brain FMO-mediated oxidation of imipramine

	K_m (μM)		V_{max} (nmoles/min/mg protein)	
	Method A	Method B	Method A	Method B
Liver microsomes	19.0	4.7	158	31
Brain microsomes	20.0	7.9	182	70

The K_m and V_{max} for FMO-mediated N-oxidation of imipramine was determined by measuring substrate-stimulated rate of NADPH oxidation (Method A) or the amount of N-oxide formed by HPLC (Method B).

TABLE 2. Effect of detergents on rat brain FMO-mediated oxidation of imipramine

	Rat brain FMO activity	
	Method A	Method B
1. – Detergent	ND	ND
2. + Sodium cholate (0.6% w/v)	108.5 ± 9.2	43.1 ± 3.5
3. + Lubrol PX (0.1% v/v)	69.7 ± 9.3	44.4 ± 6.0
4. + Triton N-101 (0.1% v/v)	75.0 ± 9.3	Not Determined
5. Freeze-thawed microsomes	77.5 ± 9.2	46.2 ± 3.7

Microsomal protein (5–20 μg) was incubated in 0.2 M Tris buffer (pH 8.5) containing 1 mM EDTA, 1 mM NADPH, 3 mM *n*-octylamine, imipramine (10 μM), and detergent as indicated. The activity was assayed either by measuring NADPH utilization rates (Method A) or by estimating the amount of imipramine N-oxide formed by HPLC (Method B). The activities are expressed as nmoles of NADPH oxidized/min/mg protein (Method A) or nmoles of imipramine N-oxide formed/min/mg protein (Method B). Values are expressed as mean ± SD (n = 4–6). ND indicates that activity was not detectable.

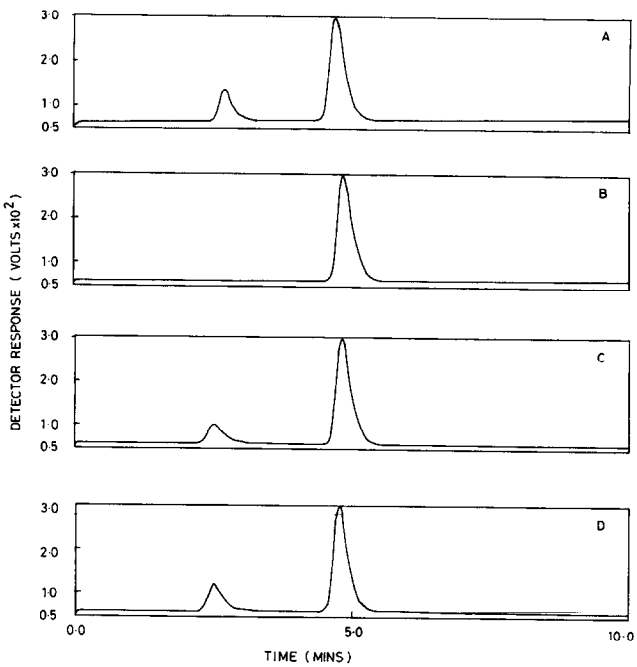

FIG. 1. HPLC elution profiles of (A) standard imipramine N-oxide and imipramine; (B) incubation performed with freshly thawed microsomes in the absence of detergents; (C) incubation performed with freeze-thawed (8 times) microsomes in the absence of detergents, and (D) incubations carried out with freshly thawed microsomes in the presence of sodium cholate (0.6%, w/v). Standard imipramine N-oxide eluted earlier than imipramine. Imipramine N-oxide was not detectable in the incubations containing freshly-thawed microsomes, although it was detectable in incubations carried out with freeze-thawed microsomes or those containing sodium cholate.

not significantly different from that measured in the presence of the cytochrome P-450 inhibitor (data not shown).

The preincubation of rat brain microsomes with antibody to rabbit pulmonary FMO prior to assay of activity resulted in total inhibition of the FMO-mediated metabolism of imipramine to its N-oxide. HPLC analysis failed to reveal the formation of any N-oxide, although the formation of the N-oxide was detectable in the microsomal incubation performed with nonimmune antibody (Table 3). However, FMO activity was inhibited by only 70.6% by the above antibody, when measured as NADPH oxidation rates (Table 3). The antiserum to rat hepatic NADPH cytochrome P-450 reductase had no effect on the FMO activity, measured using either method, indicating that there was no involvement of cytochrome P-450 in the above biotransformation pathway. The addition of an alternate substrate for FMO such as, methimazole (3 mM), resulted in inhibition of FMO activity by 86.2% and 89.5%, respectively, measured either by the HPLC method or by NADPH oxidation rates. Similar results were observed upon addition of a lower concentration of methimazole (1 mM, data not shown).

Brain FMO-mediated metabolism of imipramine was maximal when the pH of the assay buffer was 8.5. The activity declined below or above this pH (Fig. 2). Preincubation of microsomes at 45°C for 10 min resulted in the loss of 68% of the FMO activity. Activity was not detectable when the microsomes were incubated at 45°C for 20 min (Fig. 3).

A summary of the steps of purification of FMO is given in Table 4. The biological activity was monitored at each step by measuring the rate of NADPH utilization using methimazole and imipramine as substrates. The chromatography of the solubilized microsomes on *n*-octylamino-Sepharose 4B column followed by rechromatography of the flavoprotein on DEAE-Sephacel yielded a net 38-fold increase in specific activity. Elution with a linear gradient of 0 to 0.1 mM NADPH from the 2′,5′-ADP agarose column resulted in the final purification of a FMO having a net increase in specific activity of 181-fold. The specific activity of the purified rat brain FMO was 3014 nmoles of NADPH oxidized/min/mg protein for methimazole S-oxidation; the enzyme seemed to be purified only 16-fold when the activity was measured using imipramine as substrate. Interestingly, the rate of imipramine oxidation by purified FMO measured as N-oxide formed was determined to be 929 nmoles of imipramine N-oxide formed/min/mg protein (data not shown), which was not significantly higher than that measured using the NADPH oxidation rate (1125 nmoles of NADPH oxidized/min/mg protein, Table 4).

The FMO preparation was resolved into two bands on SDS-polyacrylamide gel electrophoresis (Fig. 4A), and could not be further purified even after several chromatographic procedures on 2′,5′-ADP agarose. The apparent molecular weights of the two proteins were 57.4 KDa and 61.2 KDa, respectively. By immunoblot analysis, the purified FMO cross-reacted with antiserum to rabbit lung FMO: two bands were visualized (Fig. 4B). The purified FMO did not share any immunological similarity with pig hepatic FMO as examined by immunoblot (data not shown).

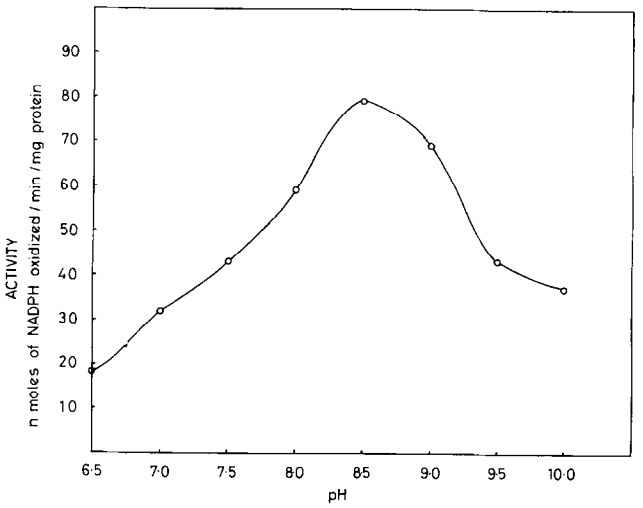

FIG. 2. The effect of pH on brain FMO-mediated oxidation of imipramine. Brain microsomes (10–20 μg) were suspended in assay buffer of varying pH (6.5–10) and the substrate (10 μM, imipramine)-stimulated rate of NADPH oxidation was measured as described in Methods. Values are the average of triplicate measurements.

TABLE 3. Inhibition of rat brain FMO-mediated N-oxidation of imipramine

	Rat brain FMO activity	
	Method A	**Method B**
1. Control	118.6 ± 18.6	44.0 ± 5.6
2. + Nonimmune goat IgG	64.3 ± 8.0	38.6 ± 2.6
3. + Antibody to rabbit pulmonary FMO	34.8 ± 4.6	Not detectable
4. + Nonimmune rabbit serum	116.6 ± 15.4	45.2 ± 3.6
5. + Antiserum to rat liver NADPH cytochrome P-450 reductase	108.5 ± 15.4	46.8 ± 5.3
6. + Methimazole (3 mM)	6.2 ± 0.5	12.5 ± 3.0

Microsomal protein (10 μg) was preincubated with immune or nonimmune antibody for 30 min at 4°C prior to the addition of 0.2 M Tris buffer (pH 8.5) containing 1 mM EDTA, 1 mM NADPH, 3 mM *n*-octylamine, sodium cholate (0.6% w/v), and imipramine (10 μM). In some experiments, methimazole (3 mM) was added along with imipramine. The activity was then assayed, either by measuring the NADPH utilization rates (Method A) or by estimating the amount of imipramine N-oxide formed by HPLC (Method B). The activities are expressed as nmoles of NADPH oxidized/min/mg protein (Method A) or nmoles of imipramine N-oxide formed/min/mg protein (Method B). Values are expressed as mean ± SD (n = 4–6).

DISCUSSION

The presence of detergents (both anionic and nonionic) in the incubation medium influenced the activity of brain

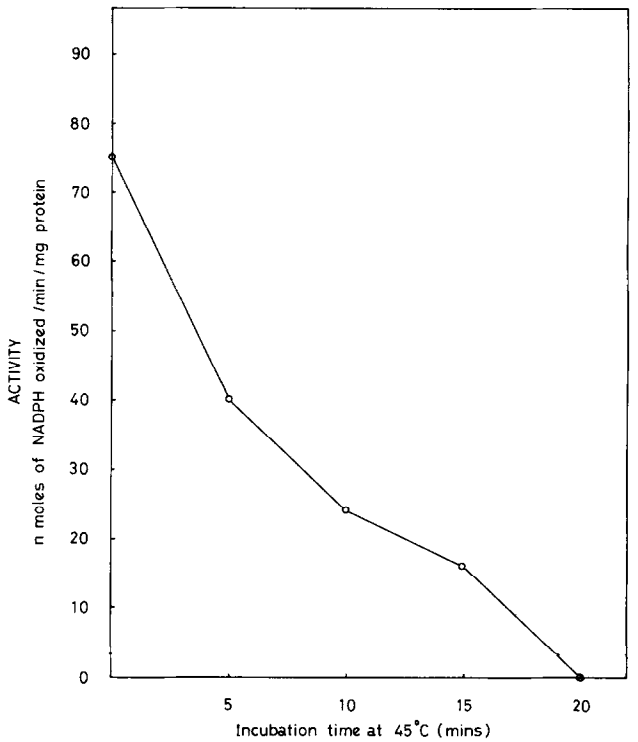

FIG. 3. The effect of temperature on brain FMO-mediated oxidation of imipramine. Brain microsomes (10–20 μg) were suspended in assay buffer (pH 8.5) and heated at 45°C for varying times (5–20 min). After the preincubation the substrate (imipramine, 10 μM)-stimulated rate of NADPH oxidation was measured as described in Methods. Values are the average of duplicate measurements.

FMO, resulting in increased enzyme activity. In the absence of detergents, negligible activity was detectable. The high lipid content of brain microsomes could be responsible for the striking detergent effects seen in the present study. The presence of detergents (or freeze thawing) may increase the accessibility of the substrate to the membrane-bound FMO. It is also possible that brain microsomes contain an inhibitor of FMO that is inactivated by detergents or freeze thawing. These observations are supported by the fact that purified brain FMO activity was not influenced by the presence of detergents. Similar effects have been observed in the FMO-mediated oxidation of methimazole and thiourea following addition of the nonionic detergent, Emulgen 911 (1% v/v), to hepatic and pulmonary microsomes, respectively [15]. The pig hepatic FMO activity is inhibited by anionic detergents like sodium cholate, but the rabbit pulmonary FMO activity is not [6]. Thus, the brain and pulmonary FMOs are similar in that the FMO activity is not inhibited by sodium cholate. However, this similarity is not reflected in the respective pH optima of the enzymes. Although the optimum pH for the pulmonary FMO is 10, the brain FMO exhibits maximal activity at pH 8.5 (Fig. 2). Our results are in agreement with those reported earlier for brain FMO [16]. Further, the pulmonary FMO is unaffected by incubation at 45°C for 10 min, in contrast to hepatic FMO, which is extremely temperature-sensitive. The brain FMO activity is partially lost under similar circumstances (Fig. 3).

The brain FMO shares immunological similarity with rabbit pulmonary FMO (FMO2) but differs functionally from FMO2. For example, FMO2 does not readily oxidize imipramine, but brain FMO efficiently metabolizes imipramine. As stated earlier, brain FMO also differs from FMO2 in the pH optimum and thermolability of the enzyme. It is, thus, possible that the brain FMO represents a unique form of the enzyme. The antibodies to FMO are not known to be immunoinhibitory; however, the rat brain FMO activity was completely inhibited by the antibody to FMO2. The reason for this unusual immunoinhibition is not known.

The traditional method of determining FMO activity has involved the measurement of the substrate-stimulated rate of NADPH oxidation. Initial measurements of brain FMO activity were carried out by determining the rate of NADPH oxidation [9]. FMO promotes a slow endogenous turnover of NADPH in the absence of substrates, forming hydrogen peroxide and $NADP^+$. To avoid the contribution to the NADPH oxidation rate by endogenous turnover, brain FMO activity has also been determined by measuring the amount of imipramine N-oxide formed by metabolism of imipramine using HPLC. The rat brain FMO activity determined in this manner is typically about 60% of that determined by measuring the NADPH oxidation rate (Tables 1 and 2). The present observations concur with those made earlier on rabbit pulmonary FMO, using *n*-octylamine and *n*-decylamine as substrates, wherein only half of the primary amine metabolized (based on NADPH oxidation rate or oxygen consumption) could be recovered as the hydroxylamine metabolite [4]. The differences observed in the present study could be due to the contribution of the endogenous turnover of NADPH, as stated before, or the NADPH dependent reduction of imipramine N-oxide back to imipramine. The FMO activity in the purified enzyme measured by the two methods did not differ significantly, suggesting that the enzyme responsible for the reduction of the N-oxide was no longer present in the purified preparation.

The rabbit pulmonary FMO is known to be closely associated with and copurifies with calreticulin [17]. To rule out the possibility of the antibody to rabbit pulmonary FMO also cross-reacting with calreticulin, we conducted immunoblot experiments with purified calreticulin and brain microsomes that were immunostained with the above antibody. The antibody to rabbit pulmonary FMO failed to cross-react with purified calreticulin, indicating that the antibody was specific to FMO (data not shown).

The purification of an FMO from brain has not been previously reported. The current study describes the partial purification of an FMO from rat brain. The presence of such an enzyme activity could conceivably contribute to the lo-

TABLE 4. Specific activity and fold purification of flavin-containing monooxygenase purified from rat brain microsomes

	Protein (mg)	Specific activity	Total activity	Yield (% recovery)	Purification (fold)
Microsomes	412.5	16.7 (69.7)	6872 (28751)	100 (100)	1.0 (1.0)
Solubilized microsomes	161.5	34.5 (117.9)	5564 (19041)	81 (66)	2.1 (1.7)
n-Octylamino-Sepharose 4B	30.4	142.7 (462.3)	4332 (14035)	63 (49)	8.6 (6.6)
DEAE-Sephacel	0.32	634 (803.7)	200 (253.2)	2.9 (0.9)	38.1 (11.5)
2,'5'-ADP agarose	0.03	3014 (1125.4)	84 (31.5)	1.2 (0.1)	181 (16.1)

The specific activity of the enzyme was assayed using methimazole or imipramine as substrate and is expressed as nmoles of NADPH oxidized/min/mg protein. Values in the parentheses indicate the activity of the enzyme using imipramine as substrate.

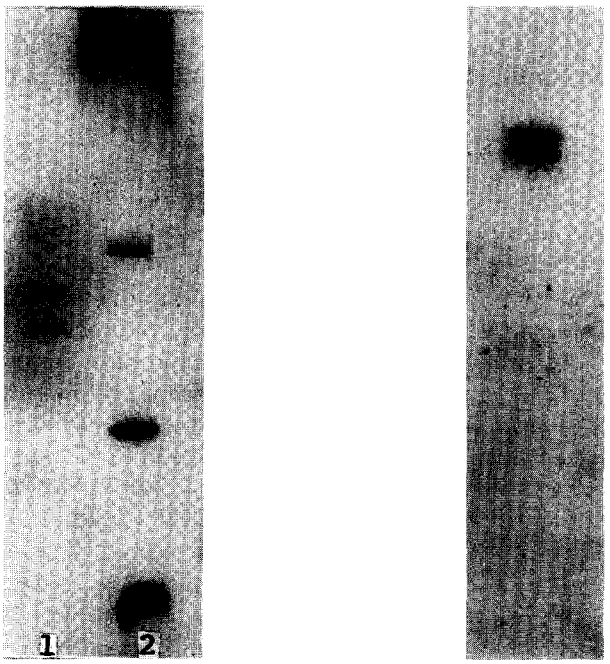

FIG. 4. (A) SDS-polyacrylamide gel electrophoresis of purified rat brain FMO and (B) immunoblot of purified FMO stained with antibody to rabbit pulmonary FMO. (A) Lane 1—Purified rat brain FMO; and lane 2—standard molecular weight markers. The amount of protein loaded was 5 μg. The gel was silver stained. The standard molecular weight markers include bovine serum albumin (66 KDa), ovalbumin (45 KDa), and pepsin (34.7 KDa). (B) The gel was loaded with 2 μg of purified protein.

cal metabolism and modulation of toxic or pharmacological effects of certain xenobiotics, including psychoactive drugs, in brain tissue.

Our attempts at purification of brain FMO yielded a preparation that was resolved into two bands on SDS-PAGE. However, both of these bands coeluted following affinity chromatography on 2',5'-ADP agarose and were immunoreactive to antibody to a pulmonary FMO. This raises two possibilities, that, 1. the lower molecular weight protein could be a proteolytic product of the higher molecular weight protein or that, 2. multiple forms of FMO exist in brain that copurify. In previous studies on intact rat brain microsomes, immunoblotting experiments using antibody to pulmonary FMO had similarly indicated the presence of two immunoreactive proteins [9]. Likewise, immunoinhibition studies, wherein the antibody to pulmonary FMO selectively inhibited imipramine N-oxidation, but not methimazole, N,N-dimethylaniline, or fluoxetine oxidation, had also indicated the possible presence of multiple forms of FMO in microsomes [9]. These observations collectively suggest the presence of multiple forms of FMO in brain, a conclusion which, however, needs further substantiation.

The specific activity of a purified rabbit pulmonary FMO for the S-oxidation of methimazole has been reported to be 573 nmoles of NADPH oxidized/min/mg protein [5]. The activity of a purified pig hepatic FMO has been reported to be 1700 nmoles of NADPH oxidized/min/mg protein [5 and Ziegler, personal communication, cited with permission]. The present activity of the purified FMO from rat brain is 3014 nmoles of NADPH oxidized/min/mg protein, which is considerably higher than that reported for the purified pulmonary and hepatic FMOs. It is interesting to note that the brain FMO was purified 181-fold when the activity was measured using methimazole as substrate, and the same protein appeared to be purified by only 16-fold when the activity was monitored using imipramine as substrate. This result suggests the possible involvement of multiple forms of FMO in the metabolism of imipramine in the brain, which could have contributed to the total activity in the microsomes.

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