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# Electrophoretic Analysis of $^3$H-Pargyline-Labeled Monoamine Oxidases A and B from Human and Rat Cells

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SUMMARY

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$^3$H-Pargyline binds specifically to both A and B forms of monoamine oxidase (MAO) under appropriate conditions. In this study the properties of $^3$H-pargyline-labeled MAO from rat and human cells were analyzed in three electrophoretic systems: sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, isoelectric focusing, and nonequilibrium pH gradient gel electrophoresis. Four types of samples were labeled with $^3$H-pargyline: crude mitochondrial preparations from rat hepatoma (with A and B activity), rat glioma (with A activity) and human fibroblasts (with predominantly A activity); and particulate fractions from human platelets (with B activity). Autoradiography of SDS–polyacrylamide gels of all four samples showed a single protein band labeled, with molecular weight of 57,000 ± 3000. Autoradiography of isoelectric focusing and nonequilibrium pH gradient gels revealed a single protein band with basic p*I*. Two-dimensional gels indicated an identity between this basic protein and the 57,000-dalton protein band. Quantitative analysis of the amount of label recovered in the specifically labeled protein band showed that binding of $^3$H-pargyline to the A form was more labile than to the B form under the conditions used for solubilization and electrophoresis in nonequilibrium pH gradient gels. These results show that A and B forms of MAO from rat and human cells have similar molecular weight and net charge, but differ in their interaction with pargyline, and suggests they represent distinct enzyme entities.

## INTRODUCTION

Monoamine oxidase (MAO,[2] monoamine: $O_2$ oxidoreductase EC 1.4.3.4.) is an important enzyme in the degradative pathway of monoamines in tissues throughout the body. On the basis of kinetic affinity for several amines and sensitivity to inhibitors, two types of activity have been described (1–3). The A type preferentially deaminates serotonin and is inhibited by clorgyline, while the B type has a stronger affinity for phenylethylamine and is more sensitive to deprenyl. Some amines, such as tyramine and tryptamine, can serve as common substrates (4); and many drugs act less selectively against these forms.

The independent distribution of the two types of activity in different tissues suggests the possible existence of two distinct isozymic forms of MAO. Biochemical and immunological characterization of the two types of activity have been hampered by the fact that the enzyme is tightly associated with the outer mitochondrial membrane (5), and its solubilization brings considerable loss of activity and alteration of kinetic properties (6, 7). A molecular distinction between the two forms was reported by McCauley and Racker (8). They prepared antibodies against a highly purified preparation of MAO from bovine liver which inhibited MAO B activity but not MAO A activity in bovine brain mitochondria. The biochemical nature of the B-specific immunogen was not described.

Recent progress has been achieved with respect to the structure of MAO using radioactively labeled suicide inhibitors such as pargyline (9–12). This drug binds specifically and stoichiometrically to MAO, forming a stable adduct with the flavin residue of the enzyme, and inhibiting its activity irreversibly (13). We have shown previously using crude mitochondrial preparations of cultured rat hepatoma cells that inhibition of both A and B types of activity by $^3$H-pargyline correlates with the labeling of a single protein band of approximate molecular weight

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[2] The abbreviation used is: MAO, monoamine oxidase.



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57,000, as determined by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and autoradiography (14). These results indicated that the two forms of MAO from rat have the same molecular weight. Similar observations were reported by Edwards and Pak (15) using mitochondria prepared from rat liver.

In the present study we examined $^3$H-pargyline-labeled MAO from rat and human cells which express A and/or B activities using isoelectric focusing, nonequilibrium pH gradient gel electrophoresis, and SDS–polyacrylamide gel electrophoresis.

## MATERIALS AND METHODS

*Cell culture and harvesting.* Rat hepatoma line $MH_1C_1$ and rat glioma line C6 were obtained from The American Type Collection (Bethesda, Md.). Diploid human fibroblast line LN BUR was obtained from a skin biopsy of a variant Lesch-Nyhan male patient (16) and was kindly provided by Dr. Uta Francke in our department.

Cells were grown as described (14, 17). Confluent cultures of hepatoma and glioma were subcultured by resuspending cells in isotonic phosphate buffered saline containing no $Ca^{2+}$ and supplemented with 1 mM EDTA. Cells were plated on 150-mm tissue culture dishes (Falcon) and fed every 2–5 days with Dulbecco's modified Eagle's medium (Grand Island Biologicals, H21) supplemented with 10% fetal calf serum (Flow) without antibiotics. Cells were harvested at confluency as described (14).

*Preparation of human platelets.* Twenty to sixty milliliters of peripheral venous blood were withdrawn from a normal female into a final concentration of 3.2 mM EDTA. All subsequent steps were carried out at 0–4° within 2–3 hr. A platelet pellet was prepared by differential centrifugation as described (18). The pellet was resuspended in a small volume of 50 mM Tris–HCl, pH 7.5 (approximately 5 mg/ml final protein concentration), and sonicated using a microprobe (Branson) at 100 W for three consecutive periods of 15 sec; the suspension was then centrifuged at 100,000*g* for 1 hr. The pellet was resuspended in 50 mM Tris–HCl, pH 7.5 (approximately 3 mg/ml final protein concentration). This particulate platelet fraction was stored in aliquots at −70°.

*Preparation of mitochondrial fractions and binding of $^3$H-pargyline.* Crude mitochondrial fractions from rat hepatoma cells, rat glioma cells and human fibroblasts were prepared as described (14). Binding of $^3$H-pargyline (6.86 Ci/mmole, New England Nuclear) to crude mitochondrial and particulate platelet fractions was performed as described (14). Binding mixtures contained 180–240 μg protein and 2 μCi $^3$H-pargyline (final concentration 0.58 μM) in 50 mM Tris–HCl, pH 7.5, in a final volume of 500 μl. Binding was carried out for 1 hr at 37°. To determine specific binding of $^3$H-pargyline to MAO A or B, preparations were incubated with clorgyline (Dr. Sabit Gabay, V.A. Hospital, Boston, Mass.) or deprenyl (Professor J. Knoll, Sammelweis University of Medicine, Budapest, Hungary), for 30 min at 37° prior to the addition of $^3$H-pargyline. Following binding, samples were centrifuged at 100,000*g* for 1 hr at 4°. Pellets were washed by resuspending in 3 ml 50 mM Tris–HCl, pH 7.5, the centrifugation repeated, and supernatants discarded.

*Electrophoretic analysis of $^3$H-pargyline-labeled proteins.* Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out by a modification of the methods of Liu and Greengard (19) and Maizel (20) as described (14), unless otherwise stated.

A modification of the method of O'Farrell *et al.* (21) was used for preparation of samples for nonequilibrium pH gradient electrophoresis. Optimal results were obtained by resuspending a pellet of $^3$H-pargyline-labeled protein (180–200 μg) in 100 μl of a solution containing 9 M urea (Schwartz/Mann), 2% Triton X-100 (Sigma), 1% β-mercaptoethanol (Sigma), 2% ampholines pH 3–10:pH 6–8, 1:1 (Bio-Rad), and 0.005% bromophenol blue. This incubation was carried out at room temperature for no longer than 10 min. After solubilization, the samples were loaded onto a slab or thin tube gel system. The slab gel was prepared as described (22), except that NP-40 was replaced by Triton X-100. The final composition of the gel was 9.2 M urea, 3.8% acrylamide, 0.2% methylene bisacrylamide, 2% Triton X-100, 0.93% ampholines pH 6–8, 1.07% ampholines pH 3–10, 0.0002% riboflavin, and 0.05% TEMED ($N_1N_1N'N'$-tetramethylethylenediamine). The slab gel was mounted on a vertical apparatus, containing 0.01 M $H_3PO_4$ in the upper tank (anode) and 0.02 M NaOH in the lower tank (cathode) (21). The current was set at 100 V for the first 15 min, 200 V for the next 15 min and 300 V for the final 3–5 hr. For isoelectric focusing protein pellets were solubilized, and electrophoresis carried out as described by Ames and Nikaido (22) in the presence of 2% ampholines pH 3.5–10:pH 6–8, 1:1.

Isoelectric focusing and nonequilibrium pH gradient gels were fixed in 10% trichloroacetic acid overnight and stained for protein as described (22). After destaining gels were treated for fluorography (23), dried and exposed to preflashed Kodak X-Omatic R film for 1–3 weeks at −70°. Quantitative distribution of radioactivity in the gels was determined by a modification (14) of the method of Horvitz (24). Radioactivity was measured by liquid scintillation spectrometry using a Beckman LS250 with counting efficiency of 52%.

*Protein determination.* Protein concentrations were determined by the method of Bradford (25) using bovine serum albumin as standard.

## RESULTS

Electrophoretic properties of the A and B forms of MAO were compared in two species: human and rat. $^3$H-Pargyline was used to label MAO in crude mitochondrial preparations from cultures of rat glioma cells (with A activity (26)), rat hepatoma cells (with A and B activity (27)) and human fibroblasts (with predominantly A activity (28, 29), and in particulate fractions from human platelets (with B activity (30)). The unbound label was removed by centrifugation and proteins were separated on the basis of molecular weight by SDS–polyacrylamide gel electrophoresis. The electrophoretic pattern of unlabeled and labeled proteins is shown in Fig. 1. For all samples, although a large number of proteins were resolved by Coomassie blue staining, only a single protein band was labeled. The molecular weight of this protein was 57,000 ± 3000 in all samples. Labeling of this protein

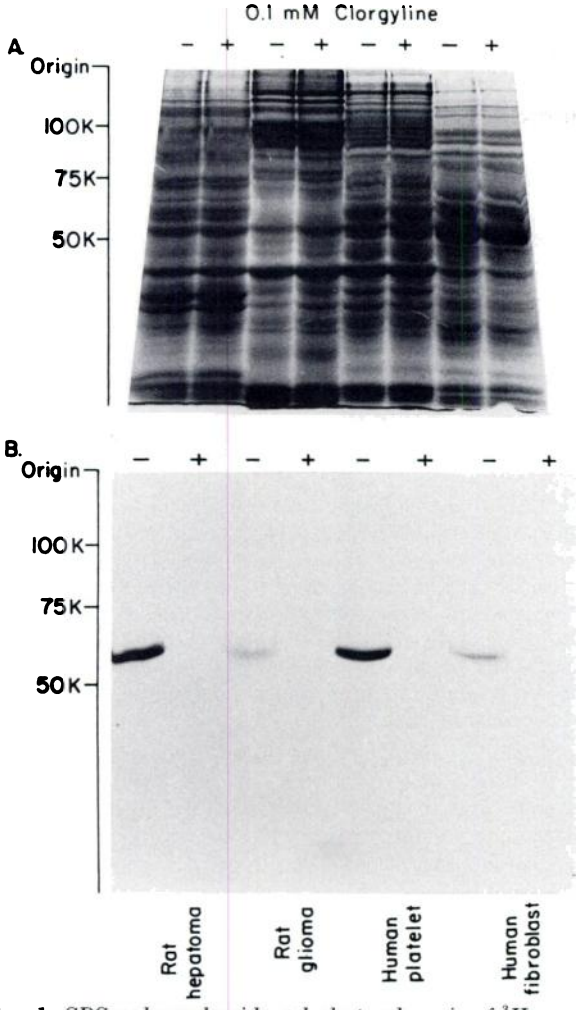


FIG. 1. *SDS-polyacrylamide gel electrophoresis of $^3$H-pargyline-labeled MAO from rat and human cells with A and B activity*

Four membrane fractions were prepared: crude mitochondria from rat hepatoma $MH_1C_1$, rat glioma C6 and human fibroblasts LN BUR, and a particulate fraction from human platelets. Suspensions of these preparations (0.18 mg protein in 0.5 ml 50 mM Tris–HCl, pH 7.5) were incubated in the absence or presence of 0.1 mM clorgyline for 30 min and then in the presence of 0.58 μM $^3$H-pargyline for 60 min. Samples were centrifuged, the pellet proteins were solubilized and SDS-polyacrylamide gel electrophoresis was carried out as described in Materials and Methods. (A) Coomassie blue staining pattern; (B) autoradiogram. The gels were treated for fluorography, dried and exposed to preflashed X-Omatic R film for 12 days at −70°. The specifically labeled band was located, sliced and counted. Radioactivity recovered was as follows: no clorgyline—rat hepatoma $MH_1C_1$ 7060 cpm; rat glioma C6 2420 cpm; human platelets 5700 cpm; human fibroblasts LN BUR 2600 cpm. No counts were detected in clorgyline-treated samples. The levels of MAO activity measured in similar preparations using $^3$H-tryptamine as substrate, expressed in nmol product/min/mg protein: rat hepatoma $MH_1C_1$ 1.7, rat glioma C6 0.32; human platelets 0.21; human fibroblasts 0.52.

band was blocked completely by preincubation with 0.1 mM clorgyline, which at this concentration inhibits both types of MAO activity (1).

If distinct proteins are responsible for the two types of MAO activity, they may differ in amino acid composition or post-translational modification, resulting in a net charge difference between them. We attempted to resolve charge differences between A and B forms of MAO by isoelectric focusing. Crude mitochondrial preparations from rat hepatoma cells were labeled with $^3$H-pargyline, solubilized and subjected to isoelectric focusing by the method of Ames and Nikaido (22). In this method, designed to improve resolution of membrane proteins, proteins are first solubilized in 2.3% SDS and then incubated in the presence of 9 M urea and 8% NP-40. This latter treatment allows proteins to dissociate from SDS, thus restoring their native charge under conditions where they remain soluble. However, in our studies solubilization of membrane proteins was not complete. As shown in Fig. 2 a considerable amount of protein, including $^3$H-pargyline-labeled protein, remained at the origin. This may have resulted from the relatively large amount of protein (200 μg/sample) that was used to improve detection of labeled proteins by autoradiography. Among the soluble proteins that migrated into the gel a single labeled protein band was detected with a p*I* of approximately 7.8. The labeling of this protein band was blocked completely by 0.1 mM clorgyline.

Isoelectric focusing of many membrane proteins requires the presence of saturating concentrations of urea as a denaturing agent, in addition to high concentrations of nonionic detergent. However, the presence of urea renders these gels particularly unstable in the alkaline pH range. We attempted a better resolution of the $^3$H-pargyline-labeled protein band in a gel system similar to isoelectric focusing but with reverse-polarity nonequilibrium pH gradient electrophoresis, as described by O'Farrell *et al.* (21). This system allows better separation of basic proteins, but fails to provide an exact determination of their net charge. $^3$H-Pargyline-labeled mitochondrial preparations from hepatoma cells were solubilized and subjected to nonequilibrium pH gradient gel electrophoresis as described under Materials and Meth-

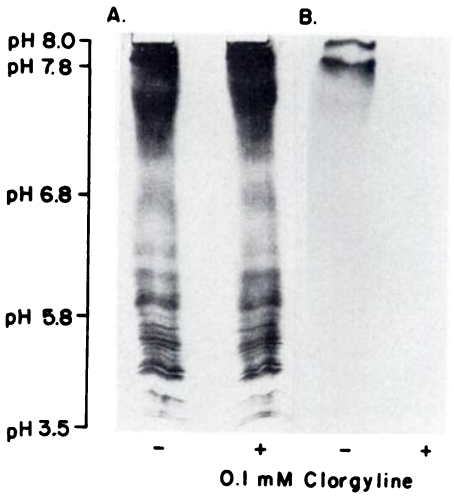


FIG. 2. *Isoelectric focusing of $^3$H-pargyline-labeled MAO from rat hepatoma cells*

Crude mitochondrial preparations from rat hepatoma $MH_1C_1$ (0.24 mg protein in 0.5 ml Tris–HCl, pH 7.5) were incubated in absence or presence of 0.1 mM clorgyline for 30 min and then in the presence of 0.58 μM $^3$H-pargyline for 60 min. Samples were centrifuged, the pellets were solubilized and isoelectric focusing carried out as described (22). (A) Coomassie blue staining pattern; (B) autoradiogram. Gels were treated for fluorography, dried and exposed to preflashed X-Omatic R film for 55 days at −70°. Protein band at p*I* 7.8 contains 410 cpm.

ods. Under these conditions solubilization of membrane proteins was more extensive than in isoelectric focusing gels but still not complete. As shown in Fig. 3, among the soluble proteins that entered the gel a single labeled protein band was detected. This protein band migrated to a position in the gel equivalent to a basic protein. Labeling of this band was blocked in a dose-dependent manner by preincubation with clorgyline (Fig. 3). Quantitation of inhibition of $^3$H-pargyline binding to this protein band by clorgyline and deprenyl is shown in Fig. 4. A comparison between drug inhibition of tryptamine deamination (27) and $^3$H-pargyline binding in crude mitochondrial preparations from these cells showed that, whereas 1 nM clorgyline blocked 80% of activity, it only blocked 33% of binding to this protein band; similarly 1 μM deprenyl blocked only 30% of activity but 83% of binding. Further, binding of $^3$H-pargyline under these conditions correlated with complete inhibition of B, but not A type activity (14). Together these inhibition studies suggest that the labeled protein band in the nonequilibrium pH gradient gels probably contains a greater proportion of the B form of MAO than was present in the original mitochondrial preparation of hepatoma cells.

$^3$H-Pargyline-labeled mitochondrial preparations from rat hepatoma cells were also examined by two-dimensional polyacrylamide gel electrophoresis. Nonequilibrium pH gradient gel electrophoresis was carried out in the first dimension in thin tube gels, followed by electrophoresis in the second dimension in SDS–polyacrylamide slab gels. A single $^3$H-pargyline-labeled protein spot was observed with an approximate molecular weight of 57,000 (Fig. 5). These results indicate that the labeled protein

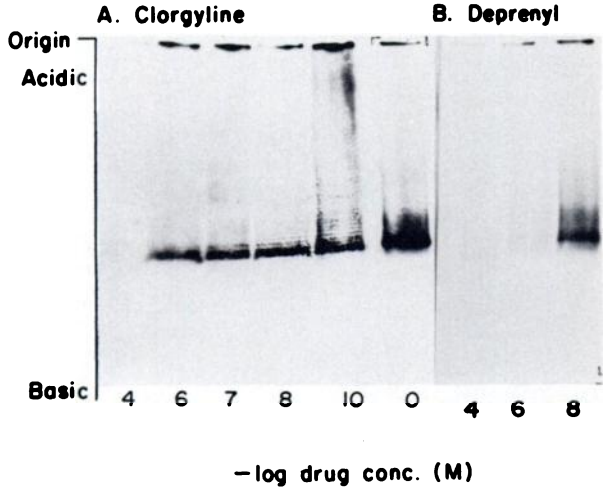


FIG. 3. *Nonequilibrium pH gradient gel electrophoresis of $^3$H-pargyline-labeled MAO from rat hepatoma cells—clorgyline and deprenyl inhibition*

Suspensions of crude mitochondria from rat hepatoma $MH_1C_1$ (0.2 mg protein in 0.5 ml 50 mM Tris–HCl, pH 7.5) were incubated in absence or presence of various concentrations of clorgyline (A) or deprenyl (B) for 30 min and then in the presence of 0.58 μM $^3$H-pargyline for 60 min. Samples were washed twice by centrifugation with 50 mM Tris–HCl, pH 7.5, pelleted proteins were solubilized and nonequilibrium pH gradient gel electrophoresis carried out as described under Materials and Methods. The gels were treated for fluorography and dried. Autoradiogram shown was obtained after exposure to X-Omatic R film for 11 days at −70°. These results are from one of five similar experiments.

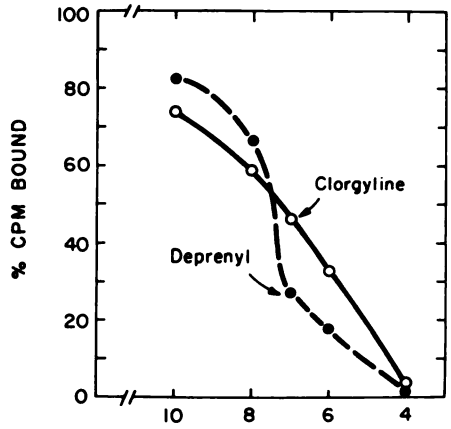


FIG. 4. *Quantitation of drug inhibition of $^3$H-pargyline binding to MAO from rat hepatoma cells*

Crude mitochondrial preparations from rat hepatoma cells (0.2 mg protein in 0.5 ml 50 mM Tris–HCl pH 7.5) were incubated in the absence or presence of various concentrations of clorgyline or deprenyl and then with $^3$H-pargyline as described in the legend to Fig. 3. Samples were prepared for nonequilibrium pH gradient electrophoresis and autoradiogram of the gels obtained as described in legend Fig. 3. The specifically labeled protein band was located, sliced and counted as described (14). Total counts present in the specifically labeled protein band from control samples, i.e., no clorgyline nor deprenyl (100% cpm bound), were 3420 cpm.

band detected in nonequilibrium pH gradient gels has the same molecular weight as the single protein band labeled in SDS–polyacrylamide gels (Fig. 1).

Nonequilibrium pH gradient gel electrophoresis was used to try to separate A and B forms of MAO from rat and human cells on the basis of charge (Fig. 6). The single labeled protein species from rat hepatoma and glioma cells, and human fibroblasts and platelets migrated to the same position. As anticipated from analysis of $^3$H-pargyline-labeled proteins in rat hepatoma cells, labeling of the A form of the enzyme, the only form present in rat glioma cells and human fibroblasts, was preferentially lost during solubilization and electrophoresis under these conditions. The extent of this loss of label was estimated as follows. Pairs of samples from each membrane preparation were labeled with $^3$H-pargyline in parallel. One sample was prepared for nonequilibrium pH gradient and the other sample for SDS–polyacrylamide gel electrophoresis. The amount of label in the specifically labeled basic protein was expressed as a percentage of the amount of label in the 57,000-dalton protein. The radioactivity recovered after nonequilibrium pH gradient gel electrophoresis was 84–88% for rat hepatoma cells, 45–57% for rat glioma cells, 75% for human platelets and 45% for human fibroblasts.

Two explanations could be offered for the low detection of the A form of MAO in nonequilibrium pH gradient gels: the A form of MAO could have a different electric charge (e.g., more acidic) than the B form; or the A form might preferentially lose its label under these conditions. We favor the latter hypothesis since (a) rat hepatoma samples incubated under conditions where both forms of MAO were labeled failed to give any labeled protein band in the acidic region of isoelectric focusing gels (Fig. 2); and (b) nonequilibrium pH gradient gels of all samples

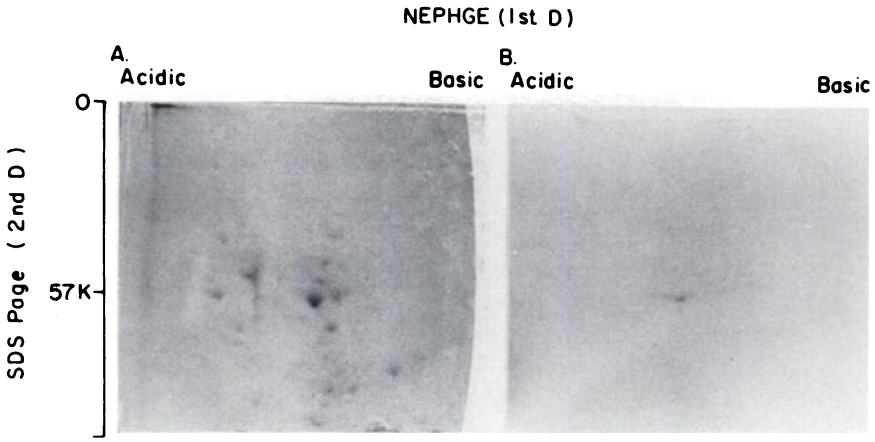


FIG. 5. *Two-dimensional polyacrylamide gel electrophoresis of $^3$H-pargyline-labeled MAO from rat hepatoma cells*

Crude mitochondrial preparations from rat hepatoma cells (0.2 mg protein in 0.5 ml 50 mM Tris–HCl, pH 7.5) were incubated in the presence of 0.58 μM $^3$H-pargyline for 60 min. Samples were washed twice by centrifugation with 50 mM Tris–HCl, pH 7.5, proteins solubilized and subjected to two-dimensional gel electrophoresis as described under Materials and Methods. (A) Coomassie blue staining pattern; (B) autoradiogram. Autoradiogram shown was obtained after exposure to X-Omatic R film for 35 days at −70°.

with only MAO A revealed a small amount of label in the same region as compared to samples with MAO B.

This difference in the characteristics of $^3$H-pargyline binding to the two forms of MAO was further investigated by examining the effects of exposure to various agents present during solubilization, prior to electrophoresis (Table 1). The amount of label bound specifically to the 57,000-dalton band was drastically reduced when the samples of rat hepatoma and glioma were boiled in SDS in the presence of 5% as compared to 1% β-mercaptoethanol prior to SDS–polyacrylamide gel electrophoresis. This loss of label was observed in all samples irrespective of what form of MAO was present. In contrast, when samples were solubilized at room temperature in the presence of 2% Triton X-100 and 9 M urea prior to non-equilibrium pH gradient gel electrophoresis, increasing the concentration of β-mercaptoethanol up to 5% had a less dramatic effect on the amount of label bound to the protein band. As noted above, the label recovered in the protein band from glioma samples was preferentially lost under conditions for solubilization and electrophoresis in nonequilibrium pH gradient gels, as compared to SDS–polyacrylamide gels. High concentrations of urea alone did not appear to be responsible for the selective loss of label from the A form. Labeled MAO from rat glioma cells showed no loss of counts when 8 M urea was present throughout solubilization and electrophoresis in SDS–polyacrylamide gels. (Higher concentrations of urea could not be tested due to precipitation of urea in the gel.)

The solubilization method described here for nonequilibrium pH gradient electrophoresis gave optimal results among a number of methods tested using hepatoma samples. When the time of solubilization was extended to 30 min, a double band of $^3$H-pargyline-labeled proteins was observed. This was interpreted to be an artifact for two reasons: (a) $^3$H-pargyline-labeled protein migrated as a double band in SDS–polyacrylamide gels with urea, but as a single band when urea was omitted; and (b) both bands of the doublet showed similar concentration-dependent inhibition by clorgyline and deprenyl.

## DISCUSSION

The biochemical differences between the two forms of MAO have been difficult to resolve due to the hydrophobic association of this enzyme with lipids and possibly other proteins in the outer mitochondrial membrane. Partial solubilization leads to aggregation and full solubilization result in loss of enzyme activity. In these studies we have analyzed the catalytic polypeptide of both forms of this enzyme by polyacrylamide gel electrophoresis under fully solubilizing and denaturing conditions. The polypeptide was labeled with an irreversible inhibitor $^3$H-pargyline *in situ* within the mitochondrial membrane prior to analysis. The specificity of labeling was determined by competition with other MAO inhibitors. The molecular weight of the catalytic polypeptides was determined by SDS–polyacrylamide gel electrophoresis. Their charge was determined by isoelectric focusing in the presence of urea and nonionic detergent. As shown in these studies MAO is a basic protein and basic membrane proteins in general have proven extremely difficult to resolve on the basis of charge. In addition to isoelectric focusing we used nonequilibrium pH gradient gel electrophoresis.

Using crude mitochondrial preparations from human and rat cells with A and/or B types of activity, we observed labeling of a single protein band at low concentrations of $^3$H-pargyline (0.6 μM). For both species and both types of MAO, this protein had a similar molecular weight of 57,000 ± 3000 and a similar charge. This suggests that the catalytic polypeptides have been conserved during evolution and that molecules responsible for A and B types of activity are very similar, but not necessarily identical in structure.

A difference between the catalytic polypeptides associated with A and B types of activity was revealed by the relative stability of the bond formed with $^3$H-pargyline. Binding of this drug to the A form was more labile than to the B form under conditions of nonequilibrium pH gradient gel electrophoresis. On the other hand binding to both forms was stable to boiling for 5 min in the

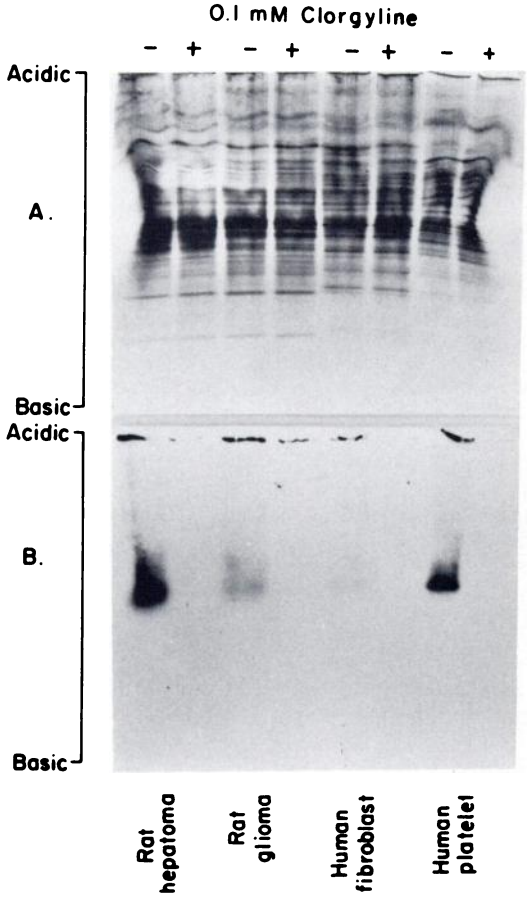


FIG. 6. *Nonequilibrium pH gradient gel electrophoresis of $^3$H-pargyline labeled MAO from rat and human cells with A and B activity*

Four membrane fractions were prepared and labeled with $^3$H-pargyline as described in legend Fig. 1. Samples were washed twice by centrifugation with 50 mM Tris–HCl, pH 7.5, proteins solubilized and nonequilibrium pH gradient gel electrophoresis carried out as described under Materials and Methods. (A) Coomassie blue staining pattern; (B) autoradiogram. Gels were treated for fluorography, dried and exposed to preflashed X-Omatic R film for 15 days at −70°. The specifically labeled protein band was located, sliced and counted as described (14). Radioactivity recovered was as follows: no clorgyline—rat hepatoma $MH_1C_1$ 3260 cpm; rat glioma C6 1440 cpm; human fibroblasts LN BUR 670 cpm; human platelets 2960 cpm. No counts were detected in clorgyline-treated samples.

presence of 3% SDS, and thus a very tight complex was formed between this drug and both forms of the enzyme. The binding of MAO suicide inhibitors such as pargyline occurs through the formation of a stable complex with the flavin residue (13) which in turn is covalently bound to the polypeptide (32). Reversal of this type of MAO inhibition has been reported for allylamine (33). The specific aspect(s) of solubilization and electrophoresis in non-equilibrium pH gradient gels which renders $^3$H-pargyline binding to the A form labile have not been resolved. The high concentration of urea (9.2 M) in the gels and migration of the enzyme through acid pH may be responsible. Alternatively, the A form may be more sensitive to secondary modifications which can occur during electrophoresis in gels containing saturating concentrations of urea (21, 31).

High concentrations of β-mercaptoethanol also reduced the amount of label recovered in the 57,000-dalton protein in SDS–polyacrylamide gels. However this effect was similar for both A and B forms of the enzyme. This loss of label could be due to reversibility of binding or to degradation of $^3$H-pargyline, the flavin cofactor or the polypeptide.

TABLE 1

*Recovery of $^3$H-pargyline-labeled MAO from SDS–polyacrylamide (PAGE) and nonequilibrium pH gradient gel electrophoresis (NEPHGE): effect of β-mercaptoethanol (β-ME ETOH)*

| Cell line (MAO type) | Gel system | Solubilization[a] | | Percentage recovery[b] |
|---|---|---|---|---|
| | | Agent | % β-ME ETOH | |
| Rat hepatoma (A + B) | SDS–PAGE | 3% SDS | 1 | 100 |
| | | | 5 | 27 |
| | NEPHGE | 2% Triton X-100, 9 M urea | 1 | 84 |
| | | | 5 | 55 |
| Rat glioma (A) | SDS | 3% SDS | 1 | 100 |
| | | | 5 | 25 |
| | NEPHGE | 2% Triton X-100, 9 M urea | 1 | 47 |
| | | | 5 | 26 |

[a] $^3$H-Pargyline labeled mitochondrial suspensions were divided into equal aliquots; each aliquot was subjected to different solubilization treatments and electrophoresis as indicated; gels were sliced and the amount of radioactivity in the specifically labeled protein band determined (see Materials and Methods).

[b] Percentage of cpm recovered in the specifically labeled protein band. Rat hepatoma, 100% corresponds to 5320 cpm; rat glioma, 100% corresponds to 2940 cpm.

In conclusion it appears that solubilized A and B forms of MAO from rat and human cells are similar in molecular weight and charge, but different in their binding to $^3$H-pargyline. The nature of the association between MAO and $^3$H-pargyline may be determined by the membrane microenvironment, which distinguishes these two types of activity, or by the intrinsic differences in molecular structure of A and B forms of the enzyme. In recent studies (34), we have observed differences among the proteolytic peptide fragments generated from the A and B forms of MAO from rat hepatoma cells. This is consistent with the hypothesis that differences between the A and B forms of MAO result from discrete differences in their amino acid sequence and/or in different post-translational modifications. The high degree of similarity between polypeptides associated with A and B types of activity favors the hypothesis that they are genetically related proteins.

## ACKNOWLEDGMENTS

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