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DIFFERENCES IN THE STRUCTURES OF MONOAMINE OXIDASES A AND B IN RAT CLONAL CELL LINES

RICHARD M. CAWTHON and XANDRA O. BREAKEFIELD*
Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

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Abstract—[^{3}H]Pargyline-labeled polypeptides associated with the A and B types of monoamine oxidase (MAO) activity in two rat cell lines were compared by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). [^{3}H]Pargyline was bound to MAO A and B in a crude mitochondrial fraction from rat hepatoma cell line MH_1C_1 and to MAO A in a mitochondrial fraction from rat glioma line C6. Specific radiolabeling of proteins associated with type A or B activity in the hepatoma samples was achieved by incubation with selective B or A inhibitors, respectively, prior to [^{3}H]pargyline binding. Following [^{3}H]pargyline binding, the samples were solubilized by heating in sodium dodecyl sulfate (SDS) in the presence of a reducing agent. SDS–PAGE of [^{3}H]pargyline bound samples revealed a radiolabeled protein band of apparent molecular weight (mol. wt) 63,000 daltons associated exclusively with MAO A activity and a band of apparent mol. wt 60,000 associated exclusively with MAO B activity. Furthermore, when SDS-solubilized, [^{3}H]pargyline-labeled MAO A and B proteins from these cell lines were subjected to limited proteolysis and one-dimensional peptide mapping in SDS gels, different patterns of [^{3}H]pargyline-labeled peptides were obtained. These findings indicate that the A and B forms of MAO activity are associated with enzyme molecules of different primary covalent structures determined by different gene loci.

Monoamine oxidase (MAO; monoamine:O_2 oxidoreductase, EC 1.4.3.4) is a mitochondrial outer membrane enzyme which oxidatively deaminates amine transmitters in the nervous system and biogenic amines throughout the body. Two types of MAO activity have been identified which differ in specificities for substrates and sensitivities to inhibitors [1, 2]. Type A activity preferentially deaminates 5-hydroxytryptamine and is more sensitive than type B activity to inhibition by clorgyline. Type B activity preferentially deaminates phenylethylamine and benzylamine and is more sensitive than type A activity to inhibition by deprenyl. Tissues from several species express MAO A alone, MAO B alone, or both types of MAO activity [3]. Several studies have addressed the question of whether the same or different enzyme molecules are associated with the two types of MAO activity.

Pargyline binds specifically and irreversibly to the flavin adenine dinucleotide (FAD) cofactor of MAO [4–6], and the FAD cofactor is covalently bound, in turn, to the MAO apoenzyme [7, 8]. Thus, after labeling cell homogenates or mitochondria with radioactive pargyline, the structures of the flavin-containing polypeptides associated with MAO A and B activities can be compared under fully solubilizing and denaturing conditions. Recently, Callingham and Parkinson [9] achieved a separation of [^{3}H]pargyline-bound polypeptides of MAO A and B from rat liver by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Similar studies in our laboratory at the time detected no differences in the mobilities on SDS gels of [^{3}H]pargyline-labeled MAO A and B from cultured rat hepatoma and glioma cell lines [10]. However, we did observe differences in [^{3}H]pargyline-bound polypeptides associated with type A and B MAO activities in these cell lines by limited proteolysis with *Staphylococcus aureus* V8 protease and one-dimensional peptide mapping in SDS-containing polyacrylamide gels [11]. These results strongly suggested that enzyme molecules with different primary covalent structures are associated with the A and B forms of MAO activity in the rat.

Here, we confirm the results of Callingham and Parkinson [9] by demonstrating differences in the mobilities on SDS gels of the pargyline-binding polypeptides of MAO A and B from rat hepatoma and glioma cell lines, and we extend our initial peptide mapping studies [11] with similar analyses using the site-specific proteases α-chymotrypsin and papain.

Crude mitochondrial preparations from a rat hepatoma line, MH_1C_1, that expresses both type A and B MAO activities [12] and a rat glioma line, C6, that expresses only type A activity [13] were incubated with [^{3}H]pargyline under conditions allowing inhibition of both types of activity. Specific radiolabeling of proteins associated with type A or type B activity in the hepatoma samples was achieved by incubation with low concentrations of deprenyl or clorgyline, respectively, prior to [^{3}H]pargyline binding. Following [^{3}H]pargyline binding, the samples were solubilized by heating in SDS in the presence of reducing agent. The SDS-solubilized [^{3}H]pargyline-bound samples were analyzed by two techniques: (1) SDS–PAGE, and (2) limited proteolysis and peptide mapping in SDS-containing polyacrylamide gels.

* Author to whom all correspondence should be sent.

MATERIALS AND METHODS

Materials and cell lines. Plastic tissue culture flasks were purchased from Falcon (Oxnard, CA); tissue culture dishes from Falcon, Corning (Corning, NY), and Nunc (Vanguard International, Inc., Neptune, IN); the Dulbecco–Vogt modification of Eagle's medium (DMEM, No. 21) and 10X Viokase from the Grand Island Biological Co. (Grand Island, NY); fetal calf serum from Flow Laboratories Inc. (Rockville, MD); and Teflon tape from W. L. Gore & Associates, Inc. (Newark, NJ). [Phenyl-3, benzyl-^{3}H]pargyline, 6.86 Ci/mmole, was purchased from the New England Nuclear Corp. (Boston, MA). Unlabeled pargyline was from Abbott Laboratories (North Chicago, IL). Deprenyl was a gift from Dr. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary. Clorgyline was from May & Baker, Ltd. (London, England). α-Chymotrypsin and papain were from Worthington (Freehold, NJ). Acrylamide, *N,N'*-methylene-bis-acrylamide (MBA), sodium dodecyl sulfate (SDS), and ammonium persulfate were obtained from Bio-Rad Laboratories (Richmond, CA), and *N,N,N',N'*-tetramethylethylenediamine (TEMED) was from the Sigma Chemical Co. (St. Louis, MO). All other chemicals used were the highest commercial grade available.

The rat hepatoma line MH_1C_1 (No. CCL 144) and the rat glioma line C6 (No. CCL 107) were obtained from The American Type Culture Collection (Bethesda, MD). Lines MH_1C_1 and C6 were derived from tumors induced chemically in the Buffalo and BD inbred rat strains respectively [14, 15].

Cell culture. Cell culture techniques were modified slightly from Hawkins and Breakefield [16]. Cells were grown as monolayers on plastic 150 mm culture dishes and 75 cm^2 flasks in 25 ml DMEM without antibiotics supplemented with 5 or 10% (v/v) fetal calf serum. Cells were kept at 37° in a humidified atmosphere of 5% CO_2 and 95% air. Cultures were fed every 5–7 days and subcultured (1:5 ratio) from a 75 cm^2 flask at confluency. To subculture, cells were rinsed twice with approximately 4 ml of phosphate-buffered saline containing 128.1 mM NaCl, 2.51 mM KCl, 7.57 mM Na_2HPO_4, 1.37 mM KH_2PO_4, 5.56 mM glucose, 58.43 mM sucrose, and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2 (buffer S); incubated for 3–10 min at room temperature with fresh buffer S; resuspended in 10 ml of culture medium by gentle trituration; and distributed to fresh culture dishes.

To harvest cultures grown on dishes, cell monolayers at confluency were rinsed quickly three times with ice-cold buffer S supplemented with 0.68 mM $CaCl_2$ and 0.81 mM $MgSO_4$ and lacking EDTA, 330 mOsM; drained for 45 sec; and scraped off the plate surface with a Teflon tape coated straight edge into 2 ml of the same buffer. Cell suspensions were frozen on dry ice and stored at −65°.

Binding of [^{3}H]pargyline. For the experiments in which radiolabeled MAO A and B were compared by analytical SDS–PAGE, mitochondrial preparations (180 μg protein/sample) were incubated with 0.18 nmoles [^{3}H]pargyline (0.36 μM) in a final volume of 500 μl of 50 mM Tris–HCl, pH 7.5 (10 mM potassium phosphate buffer, pH 7.4, is recommended for future studies), for 60 min at 37°. Following pargyline binding, samples were centrifuged at 35,000 *g* for 30 min at 4°. Supernatant fractions were discarded and pellets were stored at −65°. To identify radiolabeled proteins associated with just one type of MAO activity in rat hepatoma samples (which contain both type A and B activities), prior to [^{3}H]pargyline binding some samples (180 μg protein each) were incubated in a final volume of 490 μl of 50 mM Tris–HCl, pH 7.5, with 0.1 μM clorgyline for 30 min or 1.0 μM deprenyl for 15 min at 37° in a shaking water bath in order to block type A MAO activity or type B activity respectively. Immediately after treatment with clorgyline or deprenyl, samples were incubated with [^{3}H]pargyline, as above, collected by centrifugation, and stored at −65°.

For the experiments comparing radiolabeled MAO A and B by limited proteolysis and peptide mapping, crude mitochondrial preparations from cell lines MH_1C_1 and C6 (4 mg protein/sample) were incubated with 3.6 nmoles [^{3}H]pargyline (0.36 μM) in 10 ml of 50 mM Tris–HCl, pH 7.5, for 60 min at 37° in a shaking water bath. Some MH_1C_1 samples (4 mg protein in 9.8 ml of 50 mM Tris–HCl, pH 7.5) were incubated with clorgyline or deprenyl as described above for MH_1C_1, before addition of [^{3}H]pargyline. To test whether the inhibitors could interfere with the proteolysis of MAO, some mitochondrial preparations were incubated with clorgyline or deprenyl only after the [^{3}H]pargyline binding step. When all incubations were completed, the samples were centrifuged at 40,000 *g* for 30 min at 4°. The supernatant fractions were discarded and the pellets were stored at −65°.

Analytical SDS–PAGE. Analytical SDS–PAGE of [^{3}H]pargyline-labeled mitochondrial and platelet samples was performed using the discontinuous, high pH system of Maizel [17] as modified by Liu and Greengard [18] with additional slight modifications. The separation (resolving) gels (15 cm × 14 cm × 0.15 cm) contained 7.5% (w/v) acrylamide, 0.2% (w/v) MBA, 0.375 M Tris–HCl (pH 8.9), 0.1% (w/v) SDS, and 0.1 M sucrose, and were polymerized chemically by addition of TEMED (final concentration 0.05% [v/v]) and ammonium persulfate (final concentration 0.04% [w/v]). The stacking gels (2–3 cm × 14 cm × 0.15 cm) contained 3% acrylamide, 0.08% MBA, 0.0625 M Tris–HCl (pH 6.8), and 0.1% SDS, and were polymerized chemically by addition of TEMED (final concentration 0.05%) and ammonium persulfate (final concentration 0.8%). The electrode buffer consisted of 0.05 M Tris–HCl (pH 8.6), 0.38 M glycine, and 0.1% SDS. Radiolabeled mitochondrial pellets were resuspended in 80 μl of a loading solution containing 3% SDS, 1% β-mercaptoethanol, 3 mM EDTA, 0.15 M sucrose, 0.005% bromphenol blue, and 0.062 M Tris–HCl (pH 6.8); proteins were solubilized by heating at 100° for 4 min and then loaded into the sample wells of the stacking gel. Remaining sample wells received a mixture of standard molecular weight marker proteins in loading solution. The protein standard samples contained 2 μg of each of the following: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase (Bio-Rad); catalase

(CalBiochem); and glutamate dehydrogenase (Sigma). Electrophoresis was carried out at 45 V, constant voltage, until the tracer dye had reached the bottom of the running gel (approximately 20 hr). The SDS–PAGE procedure was essentially identical to that used by Costa and Breakefield [10] except that here the length of the resolving gel was increased by 50% and the voltage was decreased (45 V instead of 120 V). Gels were fixed and stained for protein by gentle shaking for 30 min in a solution containing 0.1% Coomassie brilliant blue R, 50% methanol, and 10% acetic acid; and destained by gentle shaking overnight in 5% methanol, 10% acetic acid [19]. Gels were prepared for fluorography using En^{3}Hance (New England Nuclear), according to the instructions from the manufacturer. The dried gels were exposed to preflashed X-Omat R Kodak film for 2–5 weeks at −65°.

Limited proteolysis and peptide mapping. [^{3}H]Pargyline-labeled MAO from rat cell lines MH_1C_1 and C6 was partially purified from mitochondrial samples using the system of SDS–PAGE described above, with the following modifications. The resolving gels were 10 cm long, and both resolving and stacking gels were 0.3 cm thick. A single, long sample well was formed in each stacking gel. Radiolabeled mitochondrial pellets (each collected after [^{3}H]pargyline binding to 4 mg protein in a 10 ml volume; see above) were solubilized in 1.6 ml loading solution as described above and loaded, one sample per gel, onto the stacking gels. Electrophoresis was performed either as above or as a shortened procedure (6 hr), in which the electrophoresis was carried out at 80 V until the tracer dye had entered the resolving gel, then continued at 120 V for the remainder of the run. After staining as above and destaining for 10 min only, a horizontal strip (approximately 10 cm × 0.3 cm × 0.3 cm) at the position in the banding pattern previously shown to contain the radiolabeled MAO [11] was removed and divided into ten 9 × 3 × 3 mm slices.

Limited proteolysis and one-dimensional peptide mapping was modified from Cawthon and Breakefield [11]. Proteolytic gels consisted of resolving gels, 10 cm long and 0.3 cm thick, identical to those described above except that they contained either 13% acrylamide/0.35% MBA (for experiments with α-chymotrypsin) or 15% acrylamide/0.4% MBA (for experiments with papain); and stacking gels, 5 cm long and 0.3 cm thick, identical to those described above except that they contained 0.125 M Tris–HCl (pH 6.8). Each proteolytic gel contained eleven sample wells, each 2.5 cm deep and 1.0 cm wide. The slices obtained by preparative SDS–PAGE and the sample wells of the proteolytic gels were separately treated with a solution containing 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, 1 mM EDTA, and 1 mM dithiothreitol (DTT) for 30 min at room temperature (10 ml/slice, and enough to fill the sample wells). The sample slices were then immediately loaded onto the proteolytic gels. Usually, three gel slices containing identically radiolabeled MAO were stacked at the bottom of each sample well. Five to six identical samples of three slices each were routinely loaded onto each gel. Sample slices, once in place, were overlaid with a solution containing 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, 1 mM EDTA, 1 mM DTT, and 20% glycerol (20 μl/well). Concentrated solutions of each protease, in 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, 1 mM EDTA, and 10% glycerol (stored at −20°), were thawed and supplemented with DTT to a final concentration of 1 mM. Serial dilutions were prepared in the same buffer and added to the sample wells. The amounts of protease added were 2, 10, 50, 200, 250, and 300 μg of α-chymotrypsin; and 0.0001, 0.0025, 0.04, 0.0625, 2.5, and 25 μg of papain. Control samples receiving no protease were also analyzed. Standard molecular weight marker proteins solubilized in loading solution (see above) were applied to two or three of the remaining sample wells. The marker proteins used were catalase (CalBiochem); ovalbumin, trypsin, myoglobin, and cytochrome *c* (Sigma); 2 μg each. Electrophoresis was carried out at a constant current of 45 mA, except for a 30-min period when the current was turned off after the tracer dye had neared the bottom of the stacking gel. Electrophoresis was continued until the bromphenol blue tracer dye in the marker lanes had moved approximately 10 cm into the running gel. The gels were then stained and destained as above, but in less than 1 hr. Each lane was cut into approximately fifty 2-mm slices and the distribution of radioactivity in the slices was determined by the method of Horvitz [20] as modified by New England Nuclear. Each slice was placed in a glass scintillation vial and 10 ml of a solution containing 5% Protosol (NEN) and 7.5% Liquifluor (NEN) (v/v) in toluene was added. Samples were incubated for 16 hr at 37°, and radioactivity was determined by liquid scintillation spectrometry. A plot of the mobilities of the marker proteins against the logarithm of their molecular weights was used to estimate the molecular weights of the radiolabeled peptide fragments.

Protein determinations. Protein concentrations were determined by the protein-dye binding assay of Bradford [21] using bovine serum albumin as standard, except that methanol was substituted for ethanol in the dye solutions.

RESULTS

SDS–PAGE of [^{3}H]pargyline-labeled proteins. Radiolabeled MAO in crude mitochondrial preparations from the rat hepatoma line was analyzed by SDS–PAGE in combination with fluorography (Fig. 1). Two radiolabeled protein bands with apparent molecular weights 63,000 and 60,000 daltons were observed (Fig. 1E). The range of molecular weight estimates for each band varied as much as 2,000 daltons among gels, but in all gels performed under these conditions the two bands were resolved by a distance equivalent to a difference in molecular weight between them of 3,000 daltons. Radiolabeling of the 63,000 dalton band was completely blocked by incubation of samples with 0.1 μM clorgyline before [^{3}H]pargyline binding, while radiolabeling of the 60,000 dalton band was unaffected by this procedure (Fig. 1D). Radiolabeling of the 60,000 dalton band was totally blocked by incubation of the mitochondrial samples with 1.0 μM deprenyl prior to [^{3}H]pargyline binding, while radiolabeling of the

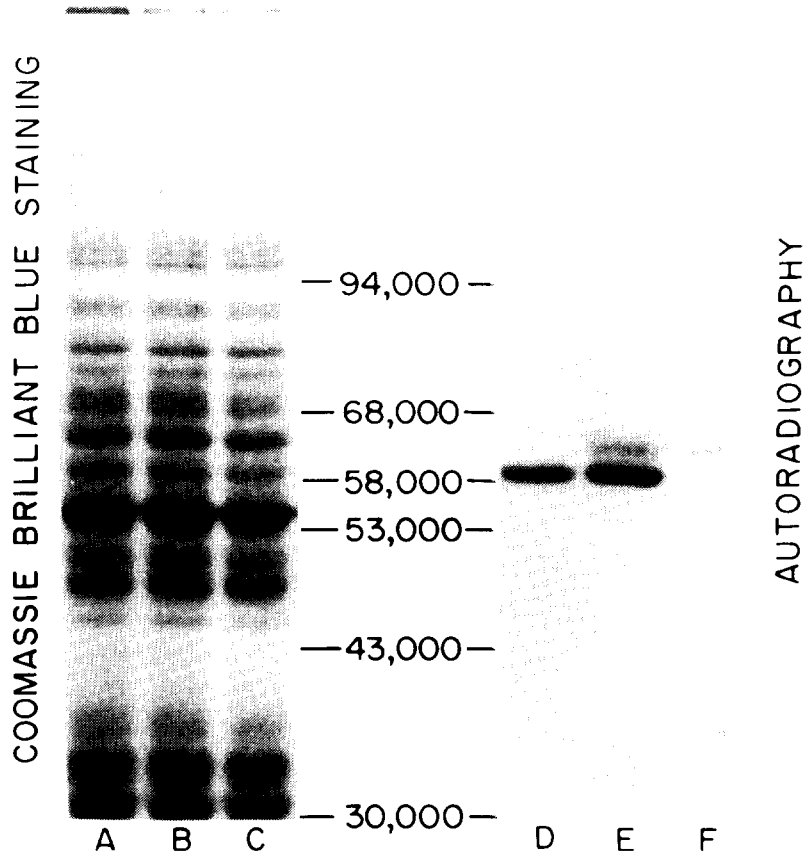

Fig. 1. SDS–PAGE of [^{3}H]pargyline-labeled crude mitochondrial fractions from rat hepatoma cell line MH_1C_1. (A–C) Coomassie brilliant blue protein staining patterns. (D–F) Fluorographic analysis of the same gel lanes. All three samples were incubated with [^{3}H]pargyline for 1 hr at 37°. Drug treatments prior to [^{3}H]pargyline binding were as follows: (D) sample incubated with 0.1 μM clorgyline for 30 min; (E) no prior drug treatment; and (F) incubation with 1.0 μM deprenyl for 15 min. See Materials and Methods for details.

63,000 dalton band was only slightly reduced (Fig. 1F). Thus, the labeled protein band at the 63,000 position was associated with MAO A activity and the labeled protein at 60,000 was associated with MAO B activity.

In other gels, [^{3}H]pargyline-labeled mitochondrial samples from the rat glioma line that expresses only type A MAO activity were analyzed along with a rat hepatoma sample (Fig. 2). Again, two labeled protein bands were obtained for rat hepatoma (Fig. 2E). The glioma samples, however, showed a single labeled band with a gel mobility indistinguishable from that of the upper band of the rat hepatoma sample (Fig. 2H). Radiolabeling of this 63,000 band was completely blocked by incubation with 0.1 μM clorgyline prior to addition of [^{3}H]pargyline (Fig. 2G) and slightly reduced by incubation with 1.0 μM deprenyl before [^{3}H]pargyline binding (Fig. 2F).

Limited proteolysis and peptide mapping in SDS gels. Site-specific proteolysis in the presence of SDS, followed by electrophoresis in SDS gels to identify peptide fragments has proven to be an effective technique for detecting differences and similarities in the primary structures of functionally related proteins of similar molecular weights [19, 22, 23]. Protease is added in a range of concentrations to a set of identical samples. The sizes of fragments obtained after gel electrophoresis are highly reproducible and characteristic for a given substrate protein and proteolytic enzyme [19]. [^{3}H]Pargyline-labeled MAO from crude mitochondrial preparations was partially purified by preparative SDS–PAGE and subjected to this peptide mapping procedure, using two different site-specific proteases: α-chymotrypsin and papain. The patterns of [^{3}H]pargyline-labeled peptide fragments obtained from MAO A and B were compared.

Figure 3 shows peptide maps obtained after limited digestion of SDS-solubilized [^{3}H]pargyline-labeled MAO with α-chymotrypsin. The single peptide map in each panel resulted from treatment of samples with a single concentration of protease, but it includes all the labeled peptide fragments that were observed when identical samples were incubated with six different concentrations of protease, and when different preparations of MAO were treated in a similar manner. The first three panels (I, II, and III) display proteolytic fragments of MAO from rat hepatoma line MH_1C_1. Panel I shows peptide fragments derived when both the A and B forms of

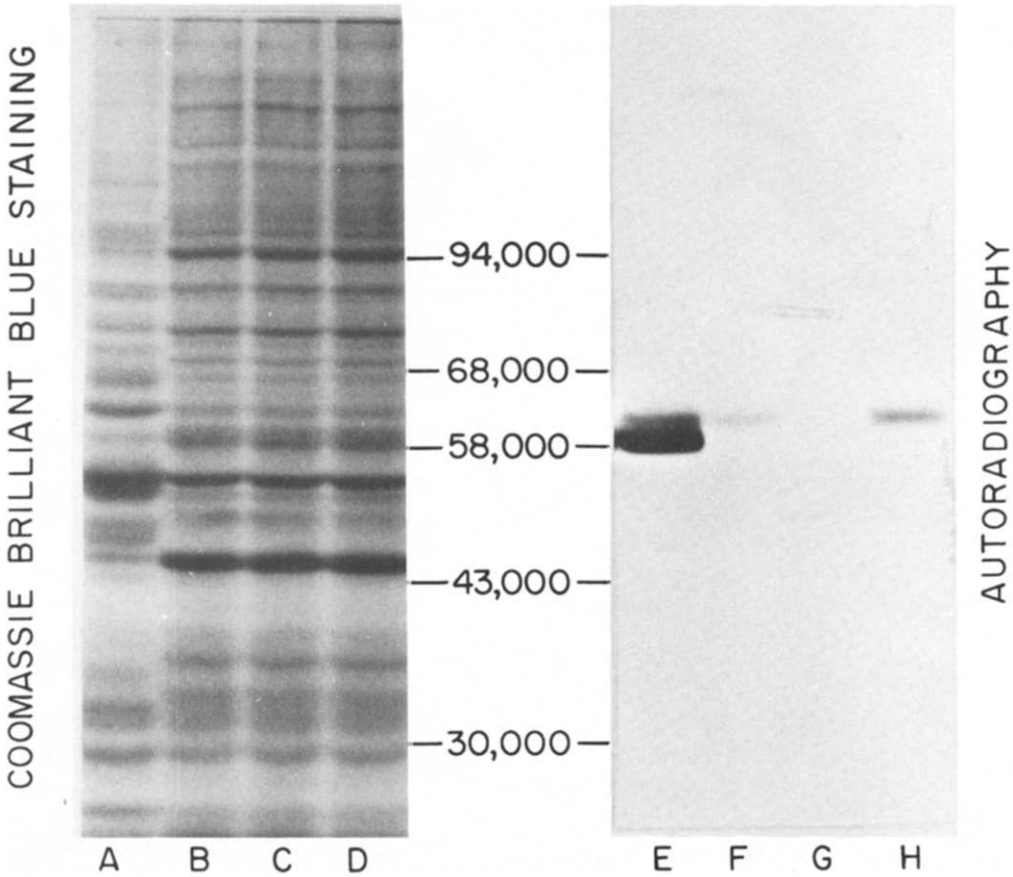

Fig. 2. SDS–PAGE of [^{3}H]pargyline-labeled crude mitochondrial fractions from rat hepatoma cell line MH_1C_1 and rat glioma line C6. (A–D) Coomassie brilliant blue protein staining patterns. (E–H) Fluorographic analysis of the same gel lanes. All four samples were incubated with [^{3}H]pargyline for 1 hr at 37°. Drug treatments prior to [^{3}H]pargyline binding were as follows: (E) hepatoma sample, no prior drug treatment; (F) glioma sample incubated with 1.0 μM deprenyl for 15 min; (G) glioma sample incubated with 0.1 μM clorgyline for 30 min; and (H) glioma sample, no prior drug treatment. See Materials and Methods for details.

MAO were labeled. Panel II shows peptides exclusively from MAO B, as binding of [^{3}H]pargyline to MAO A was blocked by preincubation of mitochondrial preparations with clorgyline. Panel III shows labeled peptides exclusively from MAO A, as binding of [^{3}H]pargyline to MAO B was blocked by preincubation with deprenyl. The [^{3}H]pargyline-labeled peptides displayed in panel IV resulted from limited proteolysis of MAO from rat glioma line C6, which expresses only type A MAO activity and was derived from a different strain of rats from the MH_1C_1 line. The leftmost peak in panels I and III co-migrated with non-proteolyzed MAO. Samples incubated with deprenyl or clorgyline only after [^{3}H]pargyline binding had peptide maps identical to those obtained for samples never exposed to these inhibitors (Panel I). Although the patterns of labeled peptides derived from MAO A and B are quite similar, the radiolabeled peptides generated from MAO A (panels III and IV) included a 38,000 dalton fragment that was missing from MAO B (panel II), and the partial digests of MAO B included fragments of approximately 23,000 and 15,000 daltons that were missing from MAO A. (Peptides unique to MAO A or B are indicated by arrows in Fig. 3.)

Proteolysis with papain produced very similar patterns for MAO A and B (Fig. 4). The two peptide maps in each panel include all the [^{3}H]pargyline-labeled fragments observed after identical samples were incubated with five concentrations of protease, and when different preparations of MAO were treated in a similar manner. In the higher molecular weight positions, three peptide peaks were shared by MAO A and B; in the lower molecular weight positions, from 15,000 to 8,000 daltons, three peaks were observed for MAO B, and only two for MAO A, but this difference was not convincing due to the close proximity of peaks.

DISCUSSION

SDS–PAGE of [^{3}H]pargyline-labeled samples from rat hepatoma and glioma cell lines resolved the flavin polypeptides of MAO into two distinct bands: a band of apparent mol. wt 63,000 daltons associated exclusively with MAO A activity and a band of apparent mol. wt 60,000 associated exclusively with

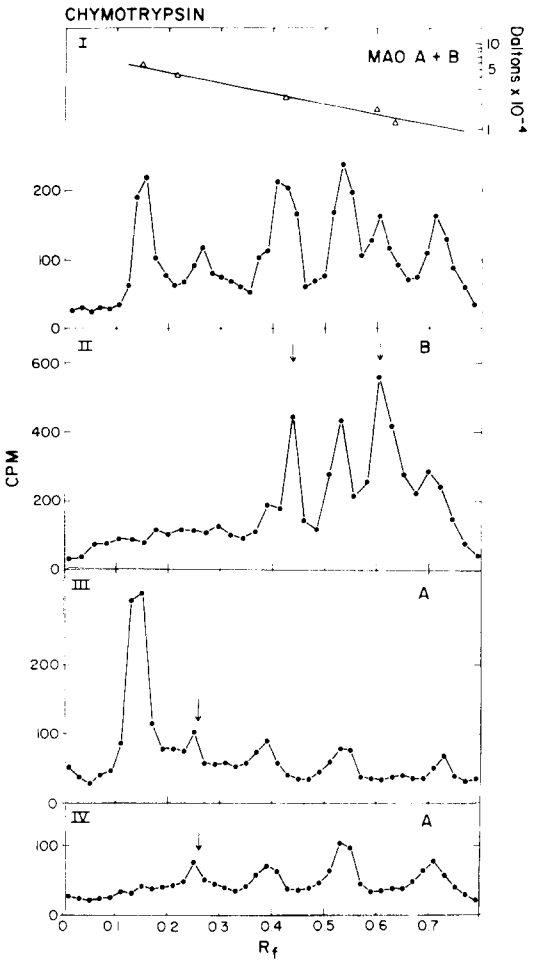

Fig. 3. Peptide maps of SDS-solubilized, [^{3}H]pargyline-labeled MAO A and B produced by limited proteolysis with α-chymotrypsin and electrophoresis in SDS-containing polyacrylamide gels. The position of slices from a gel lane relative to the tracer dye front (R_f) is plotted on the abscissa, and the radioactivity in the gel slices is plotted on the left ordinate. The graph at the top of panel I (△—△) shows the migration (R_f) of protein standards of known molecular weight (right ordinate). [^{3}H]Pargyline-labeled MAO was partially purified from crude mitochondrial fractions by preparative SDS–PAGE. Gel slices containing radiolabeled MAO were cut from the preparative gel and loaded into the sample wells of another SDS-containing slab gel (the proteolytic gel). Identical samples were loaded onto each gel and overlaid with different amounts (0, 2, 10, 50, 200, 250, or 300 μg per well) of α-chymotrypsin prior to gel electrophoresis. After electrophoresis, the distribution of radiolabeled peptides in each lane of the gel was determined by slicing the lane and quantitating the radioactivity in the slices. Panels I, II, and III show [^{3}H]pargyline-labeled peptides from line MH_1C_1: (I) mitochondrial samples received no drug treatment prior to [^{3}H]pargyline binding; (II) samples were incubated with clorgyline before [^{3}H]pargyline labeling; and (III) samples were incubated with deprenyl before [^{3}H]pargyline labeling. Panel IV shows [^{3}H]pargyline-labeled peptides from line C6: mitochondrial samples received no drug treatment prior to [^{3}H]pargyline binding. The peptide map in each panel resulted from treatment of identical MAO samples with one concentration of protease (I, 350 μg; II, 300 μg; III, 200 μg; IV, 250 μg), but includes all the peptide fragments observed when six concentrations of protease were tested. Results shown are representative of those obtained in three separate experiments using at least two different preparations of MH_1C_1 and C6 mitochondria. Control samples of MH_1C_1 incubated with clorgyline or deprenyl only after [^{3}H]pargyline labeling yielded peptide maps essentially identical to those in panel I. The leftmost peak of radioactivity in the peptide maps comigrated with intact MAO. See Materials and Methods for details.

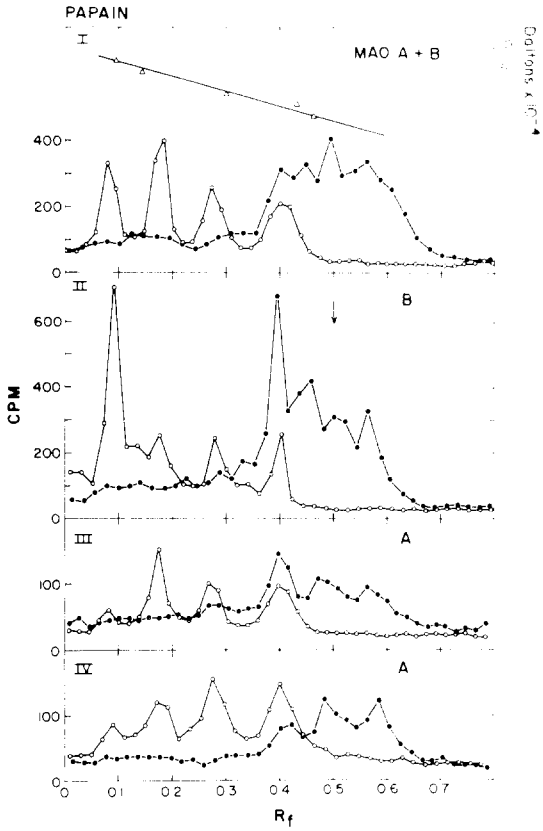

Fig. 4. Peptide maps of SDS-solubilized, [^{3}H]pargyline-labeled MAO A and B produced by limited proteolysis with papain and electrophoresis in SDS-containing polyacrylamide gels. The procedure followed was as described in Fig. 3 except that here samples loaded onto the proteolytic gels were overlaid with different amounts (0, 0.0001, 0.0025, 0.04, 0625, 2.5, or 25 μg per well) of papain prior to gel electrophoresis. Panels I, II, and III show [^{3}H]pargyline-labeled peptides from line MH_1C_1: (I) no drug treatment prior to [^{3}H]pargyline binding; (II) samples preincubated with clorgyline; and (III) samples preincubated with deprenyl. Panel IV shows [^{3}H]pargyline-labeled peptides from line C6: samples received no drug treatment prior to [^{3}H]pargyline binding. The peptide maps in each panel resulted from treatment of identical MAO samples with two concentrations of protease (I, 0.0001 and 25 μg; II, 0.0001 and 0.0625 μg; III, 0.0001 and 2.5 μg; IV, 0.0025 and 2.5 μg), but include all the peptide fragments observed with the six concentrations of protease tested. Results shown are representative of those obtained in three separate experiments using at least two different preparations of MH_1C_1 and C6 mitochondria. See Materials and Methods for details.

MAO B activity. In addition, when radiolabeled MAO samples were prepared by SDS–PAGE and subjected to limited proteolysis and peptide mapping in SDS-containing gels, different patterns of [^{3}H]pargyline-labeled peptides were generated from MAO A and B. These results strongly suggest that

the polypeptides that were examined here differ in their primary covalent structures. Furthermore, these differences in structure could not result from allelic differences at a gene locus affecting both type A and B MAO activities, since they were observed when comparisons were made between MAO A and B proteins from the same cell line. Instead, a structural difference between these proteins would have to be determined by at least one gene locus unique to one type of MAO activity. Thus, these results strongly suggest that enzyme molecules with different primary covalent structures determined by different gene loci are associated with the A and B forms of MAO activity.

In previous studies in our laboratory, identical mobilities in SDS–PAGE were found for [^{3}H]pargyline-labeled proteins of MAO A and B from these same cell lines [10, 24]. Here the resolution was improved by two alterations in the SDS–PAGE procedure: the resolving gel was lengthened, and electrophoresis was carried out at a lower voltage for a longer time. It is likely that the apparent molecular weights that we have reported for the flavin polypeptides of MAO A (63,000) and B (60,000) from rat and human [25] tissues differ from those recently reported for MAO A (60,000) and B (55,000) from rat tissues [9] and MAO A (67,000) and B (63,000) from human tissues [26] due to slight differences in the SDS–PAGE methods.

It should be noted that the distinct mobilities of type A and B proteins in SDS–PAGE may result from differences in covalent structure other than molecular weight differences. Single amino acid substitutions [27, 28], glycosylation [29], and the relative abundance and distribution of charged amino acids [30] can all extraordinarily affect the mobility of proteins in SDS gels. Similarly, the different peptide maps in SDS gels obtained for type A and B MAO proteins do not necessarily result from differences in the location of target site amino acids for the site-specific proteases, since the possible differences in covalent structure noted above could also allow a separation of peptides of equivalent size, and/or could alter the susceptibility of a target site to cleavage. The effects of other membrane components on proteolysis of MAO have been eliminated by denaturing the enzyme and solubilizing it in SDS prior to proteolysis (see Ref. 25 for further discussion).

Here, only one type of MAO A protein was identified by analytical SDS–PAGE of [^{3}H]pargyline-labeled samples from two rat cell lines derived from different tissues and different strains of rats. Furthermore, limited proteolysis and one-dimensional peptide mapping in SDS gels, using three different site-specific proteases, detected no structural differences in these MAO A proteins ([11], and results presented here). However, differences between [^{3}H]pargyline-bound MAO A proteins from mouse and human cell lines have been detected by this technique [31], and it is also possible that differences in the structure of proteins associated with type A or type B activities may occur in different tissues from the same strain of rats and in the same tissue from different strains. Further analyses of MAO by two-dimensional peptide mapping and other sensitive techniques are needed to test these possibilities.

In conclusion, SDS–PAGE and peptide mapping of [^{3}H]pargyline-labeled MAO from two rat cell lines have identified two distinct flavin polypeptides: one associated exclusively with type A MAO activity, the other associated exclusively with type B activity. These results confirm and extend recent reports demonstrating structural differences in SDS-solubilized, [^{3}H]pargyline-bound polypeptides of MAO A and B from rat liver [9] and two rat cell lines [11]. Similar results have been reported recently for MAO A and B from human tissues [25, 26]. Thus, of those gene loci affecting the structure of the flavin-containing polypeptides, there must be at least one such locus specific to one type of MAO activity. Type A and B proteins could be determined by separate structural gene loci that code for different amino acid sequences. Alternatively, the two proteins could result from differences in the modification or processing of a common initial RNA transcript coded by a single structural gene locus. This mechanism would require one or more additional loci to control differential post-transcriptional processing. Finally, type A and B proteins could arise from differences in post-translational covalent modification(s) of a common enzyme precursor coded by a single structural gene locus. Again, one or more additional loci would be required to control the differential post-translational modification(s). Further work will be necessary to determine which of these explanations is correct.

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