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SHORT COMMUNICATIONS

Monoamine oxidase: separation of the type A and B activities

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Monoamine oxidase (MAO*, EC 1.4.3.4) is an integral outer-mitochondrial membrane enzyme known to exist in at least two functionally and structurally distinct forms, MAO-A and B [1–5]. Although the existence of the A and B forms of MAO has been known since the work of Johnston [3] in 1968, the application of various chromatographic techniques has not provided unequivocal evidence that the oxidases can be physically separated as catalytically active species which also retain native enzymatic properties. Recently, White and Stein [6] have suggested that the two forms of the oxidase may be held tightly together as subunits of a phospholipid-containing structure within the membrane. Based on kinetic arguments, Fowler and Oreland [7] have proposed that the two forms of MAO not only exist in close proximity within the outer-mitochondrial membrane but may also catalytically interact. These reports suggest that the two oxidases are closely associated within the outer-mitochondrial membrane in a manner which prevents separation by nonenzymatic techniques. In contrast, the data presented in this manuscript demonstrate that both the A and B forms of human brain MAO can be separated by ion-exchange chromatography after solubilization with octylglucoside (OG).

Materials and methods

Radioactively labeled [^{14}C]-5-hydroxytryptamine (5-HT) binoxalate (49.3 mCi/mmole), [^{14}C]-phenylethylamine (PEA) hydrochloride (48.3 mCi/mmole) and [^{14}C]-tyramine (TYR, 55.3 mCi/mmole) were purchased from the New England Nuclear Corp., Boston, MA. Unlabeled amines and pargyline were obtained from the Sigma Chemical Co., St. Louis, MO. Octylglucoside was purchased from Calbiochem, La Jolla, CA, and clorgyline was a gift from May & Baker, England.

MAO was assayed by the procedure described previously [8]. To determine the percent inhibition by clorgyline, enzyme was preincubated for 30 min at 37° in the presence of various concentrations of clorgyline prior to addition of substrate.

Human brain frontal lobes were obtained at autopsy within 12 hr after death, and crude mitochondria were isolated from this tissue as described previously [9]. For solubilization of MAO, mitochondrial preparations (5 mg protein/ml) in 0.1 M potassium phosphate buffer, pH 7.4, were stirred gently with 50 mM OG at 0° for 30 min. The mixture was centrifuged at 100,000 *g* for 60 min, and the supernatant solution was dialyzed overnight against a total of 100 vol. of buffer with one change of buffer after 3 hr. The dialysate obtained following solubilization was centrifuged at 100,000 *g* for 60 min, and the precipitate was resolubilized by homogenization in 50 mM triethanolamine buffer, pH 7.4, containing 100 mM OG. This solution was immediately added to a DEAE-Sepharose CL-6B column (1.0 × 28 cm) pre-equilibrated with 50 mM triethanolamine buffer, pH 7.4, containing 30 mM OG, and the enzymes were eluted with a 200 ml NaCl gradient (0 to 0.2 M). Fractions (3 ml) were collected and assayed for 5-HT and PEA deaminating activity. Following chromatography, samples were pooled from each peak, as indicated in Fig. 1, and dialyzed overnight against 0.1 M potassium phosphate buffer, pH 7.4. The dialysates were then centrifuged overnight at 250,000 *g*, and the pellets were resuspended in phosphate buffer by homogenization. Protein concentrations were determined by the method of Lowry *et al.* [10]. Phosphate was determined by the method of Bartlett [11].

Results and discussion

The elution pattern obtained from DEAE-Sepharose CL-6B chromatography of OG-solubilized human brain MAO is shown in Fig. 1. The data indicate that PEA deaminating (B-oxidase; peak 1) activity eluted prior to 5-HT deaminating (A-oxidase; peak 2) activity, which eluted at higher salt concentrations. As shown in Table 1, solubilization and ion-exchange chromatography resulted in approximately a 19-fold purification of the enzyme based on the activity associated with peak 1 and 15-fold purification of the A enzyme activity associated with peak 2.

Pooled fractions from peaks 1 and 2 were also evaluated for percent enrichment of either the A or B forms of MAO resulting from their chromatographic separation. As illustrated in Fig. 2, the selective MAO-A irreversible inhibitor, clorgyline, was used to determine the relative contribution of the A and B forms of MAO based on TYR deaminating activity in both the native (mitochondrial) preparation and the pooled fractions obtained following ion-exchange chromatography. As expected, a biphasic curve was obtained with the mitochrondrial preparation, revealing that TYR was deaminated by both the A and B forms of the oxidase. These results demonstrate that under the conditions employed approximately 60% of TYR deamination is contributed by the B form of the oxidase and the remaining 40% by the A form of MAO. Clorgyline inhibition of TYR deamination associated with peak 1 gave a simple sigmoidal curve, whereas inhibition of the activity associated with peak 2 produced a biphasic curve. These data reveal that the sample from peak 1 contained exclusively the B form of the oxidase, whereas that from peak 2 contained both forms of the oxidase with 70% of TYR deamination contributed by the A form and the remaining 30% by the B form of MAO. This represents almost a 2-fold enrichment of the A form of the oxidase in this latter fraction as compared to that in the starting mitochondrial preparation.

The results of ion-exchange chromatography indicate that the A and B forms of MAO can be separated on the basis of charge; however, the molecular composition of the solubilized enzyme species is not known. Although Breakefield and coworkers [5] have demonstrated that the two forms of MAO are structurally distinct, the difference in charge of these two detergent-solubilized entities may also be a consequence of specific components of the membrane microenvironment of the two enzymes, particluarly phospholipids, which remain associated with the oxidases following the solubilization procedure. In contrast to our findings, White and coworkers [12, 13] reported that SDS-treated preparations of MAO obtained from rat heart

* Abbreviations: MAO, monoamine oxidase; OG, 1-*O*-*n*-β-D-glucopyranoside (octylglucoside); 5-HT, 5-hydroxytryptamine; PEA, phenylethylamine; TYR, tyramine; and SDS, sodium dodecyl sulfate.

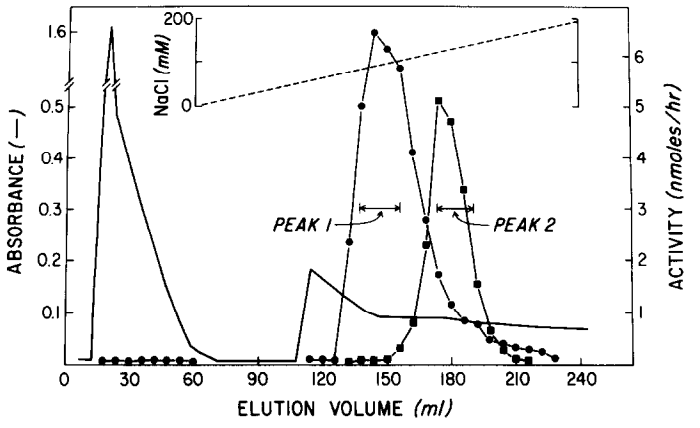

Fig. 1. Chromatographic separation on a DEAE-Sepharose CL-6B (1.0 × 28 cm) column of human brain type A and B monoamine oxidase. Enzyme was eluted with a 200 ml NaCl gradient (0 to 0.2 M) in the presence of 50 mM triethanolamine buffer, pH 7.4, and 30 mM octylglucoside. Deamination of phenylethylamine (4.6 μM) (●—●) and 5-hydroxytryptamine (100 μM) (■—■) was measured at 37° for 10 and 60 min respectively. Protein elution was followed by the change in absorbance recorded at 254 nm.

(MAO-A) and human platelets MAO-B) were not separable by electrofocusing. However, the extent of migration of the two forms of MAO in these gels may have been largely due to SDS remaining bound to both enzyme species. Jain and Sands [14] found that solubilized human brain MAO produced a single band of activity when subjected to polyacrylamide electrophoresis. This finding was carried out in the absence of detergent where such conditions would be expected to promote aggregation of membrane proteins producing the observed results.

McCauley and Racker [15] reported that the two forms of MAO from bovine liver and brain could be separated on the basis of selective precipitation by antibodies directed against the B oxidase. The B enzyme activity which precipitated was very unstable at 37° and required characterization at 4°. Denney *et al.* [16] demonstrated that the B form of MAO could be selectively removed from an octylglucoside-solubilized enzyme preparation containing both forms of the oxidase by means of immunoaffinity column chromatography. However, the B form of the oxidase was inactivated in the process of the separation on chromatography.

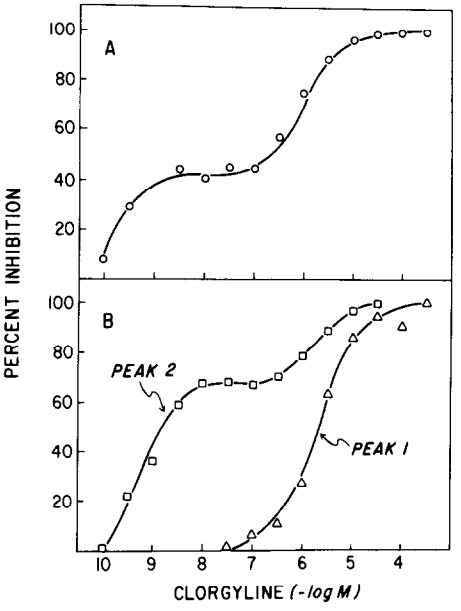

Fig. 2. Clorgyline inhibition of tyramine (100 μM) by native membrane-bound MAO (○—○) and the fractions pooled from peaks 1 (△—△) and 2 (□—□) obtained on DEAE-Sepharose CL-6B chromatography. The appropriate enzyme preparation was preincubated for 30 min at 37° in the absence or presence of various amounts of clorgyline in 0.05 M potassium phosphate buffer, pH 7.4, prior to addition of tyramine. The reaction was continued for an additional 30 min and terminated with addition of 0.4 M HCl. Assays were initiated by the addition of enzyme and contained 0.12 mg protein of mitochondrial enzyme or 4 μg protein from peak 1 and peak 2 samples.

Table 1. Purification of monoamine oxidase by ion-exchange chromatography on DEAE-Sepharose CL-6B*

Enzyme preparation	% Yield A	% Yield B	Purification A	Purification B
Mitochondria	100	100		
OG-treated	72	73	3.6	2.4
Chromatography pooled fractions				
46–52	15	5	15	4
58–63	0	30		19

* Deamination of 100 μM 5-hydroxytryptamine (MAO A) and 4.6 μM phenylethylamine (MAO B) was assayed at 37° for 60 and 10 min respectively. Protein concentrations in the assay were 0.12 mg for the mitochondrial enzyme, 0.04 mg for the octylglucoside-treated enzyme, or 4 μg for the chromatographed enzyme in a total volume of 0.4 ml. Yields of A and B enzyme activity obtained following chromatography were determined for dialyzed pooled samples from peaks 1 and 2 respectively. Specific activities for 5-HT and PEA deamination in mitochondrial fractions were 0.86 and 0.80 nmoles per mg protein per min respectively.

Recently, Denney *et al.* [17] reported that the B form of either human platelet or liver MAO could be selectively precipitated, with retention of enzymatic activity, using a monoclonal antibody to human platelet MAO-B. These latter findings along with those reported herein demonstrate that the two forms of the oxidase can indeed be physically separated by either immunological or simple chromatographic techniques. These findings are also consistent with the hypothesis that the two forms of MAO are, in fact, different proteins; however, the possibility that other factors may account for these differences cannot as yet be ruled out.

As briefly indicated in the introduction, White and Stein [6] have proposed recently that the two oxidases are embedded in the same membrane complex and that this complex in human liver and brain can be disrupted following treatment with phospholipase A2 and/or sodium dodecyl sulfate. Their results demonstrated that the two activities were not clearly separable by electrophoretic or gel filtration techniques. In contrast, the findings presented in this paper indicate that separation of the human brain oxidases occurs under conditions which are relatively mild [18] and would not be expected to be vigorous enough to disrupt the proposed complex. Additionally, the lipid to protein ratio of the enzyme eluting from the ion-exchange column was found to be 0.8 which is very similar to the lipid to protein ratio observed in the outer-mitochondrial membrane [19]. Agreement between the literature value for the native lipid to protein ratio and that found in the partially purified preparation of MAO further supports the hypothesis that treatment with octyglucoside, at the concentrations employed herein, does not disrupt significantly the native lipid–protein interactions.

In summary, these results constitute the first report which demonstrates that the oxidases are separable as catalytically active species by simple chromatographic techniques. These findings do not support the concept that the A and B forms of MAO are held tightly together in a membrane complex which prevents separation of the activities. Furthermore, the inability of previous authors [12, 13] to separate the A and B forms of MAO may be more dependent on the properties of the detergent used to solubilize and chromatograph these proteins than on the state in which these proteins exist within the outer-mitochondrial membrane.

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State University of New York at Buffalo
School of Medicine
Department of Pharmacology and Therapeutics
Buffalo, NY 14214, U.S.A.

L. BRUCE PEARCE
JEROME A. ROTH*

* Send correspondence to: Jerome A. Roth, Ph.D., Department of Pharmacology and Therapeutics, School of Medicine and Dentistry, 127 Farber Hall, State University of New York at Buffalo, Buffalo, New York 14214.

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Regional differences in adrenocortical benzo[*a*]pyrene metabolism in guinea pigs

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The extrahepatic metabolism of xenobiotics has been studied extensively in recent years, in part because of the potential for the production of reactive metabolites, resulting in locally toxic effects. Polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene (BP), are among the compounds whose adverse effects depend upon activation by microsomal enzymes (see Refs. 1–3). We previously demonstrated high rates of BP metabolism by microsomal preparations from guinea pig adrenal glands [4, 5], and BP is also rapidly metabolized by human fetal adrenals [6, 7]. There is an anatomical zonation of the adrenal cortex [8], and different roles in steroidogenesis have been attributed to each zone. However, relatively little is known about regional differences in the metabolism of xenobiotics within