The Insertion of Monoamine Oxidase A into the Outer Membrane of Rat Liver Mitochondria*

Zhengping Zhuang, Bernard Marks, and Roy B. McCauley

From the Department of Pharmacology, Wayne State University, Detroit, Michigan 48201

Human monoamine oxidase A that had been synthesized in a reticulocyte lysate translation system was capable of binding to and inserting into either rat liver mitochondria or isolated mitochondrial outer membranes. The inserted form was as resistant to proteinase K as endogenous mitochondrial monoamine oxidase A. The insertion, but not the binding, of monoamine oxidase A was prevented by depleting the reaction mixture of either ATP (with apyrase) or ubiquitin (with purified antibodies against this polypeptide). Addition of ATP or ubiquitin, respectively, to these depleted mixtures restored the insertion of the enzyme.

In the absence of mitochondria, in vitro synthesized monoamine oxidase A did not catalyze its own alkylation by the mechanism-based inhibitor, [3H]clorgyline. However, both monoamine oxidase A that had been membrane-inserted in vitro and monoamine oxidase A that had been bound to the mitochondria under conditions of ATP depletion catalyzed adduct formation. Furthermore, reaction of either clorgyline or another mechanism-based inhibitor, pargyline, with the membrane-bound enzyme during ATP depletion inhibited the insertion of monoamine oxidase A when ATP was restored. These observations indicate that monoamine oxidase A acquired a catalytically active conformation on interaction with the mitochondrial outer membranes prior to its ATP and ubiquitin-dependent insertion into the membrane.

Monoamine oxidase A and monoamine oxidase B are integral proteins of the outer membrane of mammalian mitochondrial (1, 2). The monoamine oxidase isoenzymes have different substrate specificities, are immunologically distinct and have different primary structures (3, 4). Monoamine oxidase A and monoamine oxidase B also can be distinguished by their ability to oxidize and form adducts with the mechanism-based inhibitors clorgyline and pargyline (5). These adducts form with the covalently linked flavin cofactor that is present in the isoenzymes (6). Monoamine oxidase A is more sensitive to clorgyline than to pargyline, whereas monoamine oxidase B is more sensitive to pargyline.

Most studies of the assembly of proteins into the outer membranes of mitochondria have been done in vitro using yeast or Neurospora porin, the outer membrane pore-forming protein. Porin is thought to be bound to a "receptor" protein on the outer membrane surface prior to its insertion. This 19-kDa protein, MOM 19, also binds other protein precursors that are imported into the interior of the mitochondria (7, 8). Porin has been shown to insert into yeast mitochondria and isolated outer membranes by a process that does not require a membrane potential (9); however, in vitro synthesized porin requires extramitochondrial ATP for translocation into isolated mitochondria (10, 11). On the other hand, porin purified from Neurospora mitochondria and treated with acid and alkali becomes "water-soluble." This porin form will insert into mitochondria to form functional pores in the absence of ATP (11). These observations and others (12, 13) suggest that ATP may be involved in the conformational changes that occur during translocation of many proteins into or across mitochondrial membranes.

Less is known about the insertion of proteins into mammalian mitochondrial outer membranes. In vitro synthesized mammalian porin has been reported to insert into rat liver mitochondria (14) but, unlike yeast porin, not into isolated outer membranes prepared from these mitochondria (15). Monoamine oxidase B, on the other hand, has been shown to insert into both rat liver mitochondria and their isolated outer membranes (16). Monoamine oxidase B insertion requires ATP and ubiquitin (17), a 76-amino-acid polypeptide that is involved in a number of cellular functions, including a role in the heat shock response, ribosome biogenesis, and protein degradation (18-20). In the work presented here, monoamine oxidase A also has been shown to bind to and insert into rat liver mitochondria and isolated outer membranes. Both ATP and ubiquitin were required for insertion. Monoamine oxidase A also acquired catalytic activity upon binding to mitochondria, prior to its insertion into the mitochondrial outer membranes. These observations indicate that at least some domains of this membrane-bound form of monoamine oxidase A were very similar to the membrane inserted form before the ATP- and ubiquitin-dependent insertion reaction occurred.

**Experimental Procedures**

Preparation of Mitochondria and Isolation of Mitochondrial Outer Membranes—Mitochondria and mitochondrial outer membranes were prepared from rat liver, as described previously (21). The outer membrane preparation is a variation of the osmotic method first described by Sottocasa et al. (1). Both mitochondria and outer membranes were stored frozen at 70 °C at 10-20 mg of protein/ml in 0.3 M sucrose containing 10 mg/ml defatted bovine serum albumin (Sigma). Both were washed once prior to their use by dilution in 15 volumes of 0.3 M sucrose and centrifugation at 14,000 × g for 10 min or 100,000 × g for 60 min.

In some experiments, suspensions of 10 mg/ml mitochondrial protein were preincubated for 60 min at 25 °C with 1 mM each of clorgyline and pargyline to inhibit endogenous monoamine oxidase activities. These mitochondria were washed five times by resuspension and centrifugation using 20 volumes of 0.3 M sucrose for each wash. Finally, the mitochondria were stored frozen (as above) until use.

In Vitro Synthesis of Monoamine Oxidase A—RNA for monoamine oxidase A synthesis was prepared by transcription of human cDNA
avoid extraneous transcripts due to a 3' overhang (22). Transcription was precipitated at -20 °C with times with 70% ethanol, and stored up to 5 days in 100% ethanol. Assay volumes were 50-200 μl in volume. The reticulocyte lysate was incubated for 90 min in a reaction mixture that contained 0.5 mM ATP, CTP, and PstI, lysates and other materials obtained commercially (Promega Biotec). ATP, creatine phosphate, creatine phosphokinase, and a buffered salt mixture. Mitochondria or isolated outer membranes were always held to an ice bath. When the binding of monoamine oxidase A was estimated, the mitochondria were collected by centrifugation at 14,000 g for 10 min (outer membranes were centrifuged at 100,000 g for 60 min at 4 °C) and assayed by SDS-PAGE and fluorography. When the insertion was measured, the reaction was treated with 10 μg of proteinase K for 15 min on ice before the mitochondria or outer membranes were isolated from the mixture. This treatment was followed by the addition of 2 mM PMSF, and the mixture was held on ice for 5 min more before centrifugation and analysis as above. In instances when ATP was depleted, the ATP-regenerating system was omitted, and the translation mixture was treated with 1 unit of apyrase (Sigma)/ml for 20 min at 25 °C after the synthesis of [35S] monoamine oxidase was completed but prior to its addition to the reaction mixture. In instances when ubiquitin was depleted, the translation mixtures containing [35S] monoamine oxidase were treated for 60 min at 25 °C with either purified IgGs from antiubiquitin (25) or preimmune sera (each 500 μg/ml). These IgG-treated mixtures were added to reaction mixtures that contained the ATP-regenerating system. IgGs were purified from S. aureus protein A immobilized on agarose beads (Sigma), as has been described (26). Each of the experiments presented here is typical of the results of at least three experiments.

The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

### RESULTS

#### The Insertion of Monoamine Oxidase A into the Outer Membranes of Mitochondria

The endogenous monoamine oxidase activities of rat liver mitochondria have been shown to be protected by the outer membrane from treatment with proteinases (2), and in a previous study (16), it had been shown that protection from proteinase K could be used as an assay for the insertion of in vitro synthesized monoamine oxidase B into mitochondria or isolated mitochondrial outer membranes. Experiments were performed to determine whether a similar method could be used to assess the in vitro insertion of monoamine oxidase A. Rat liver mitochondrial membranes were incubated at 4 °C for 15 min (under the conditions described in Fig. 1) with increasing concentrations of proteinase K, and the reaction was stopped with 2 mM PMSF. The mitochondria were isolated by centrifugation, and reacted with 5 μM [3H]clorgyline. Clorgyline is a mechanism-based monoamine oxidase A inhibitor that must be oxidized by the enzyme before it can form an adduct with the covalently linked monoamine oxidase A flavin (6). Adduct formation is, therefore, not only a means for identifying monoamine oxidase A but also an indication of monoamine oxidase A activity. After the reaction with [3H]clorgyline, the mitochondria were analyzed by SDS-PAGE and fluorography. As can be seen from Fig. 1A, endogenous monoamine oxidase A retained enzymatic activity after treatment with concentrations of proteinase K of 10 μg/ml or less. Furthermore, these concentrations of proteinase K had no detectable effect on the electrophoretic mobility of monoamine oxidase A, suggesting that the enzyme had apparently not been cleaved in domains that did not affect catalysis. On the other hand, when a

![Panel A](image1.png) ![Panel B](image2.png) ![Panel C](image3.png)

**Fig. 1.** The effect of proteinase K on endogenous mitochondrial monoamine oxidase A and in vitro synthesized monoamine oxidase A. Panel A, 100 μg/ml rat liver mitochondria were incubated at 37 °C for 30 min under the conditions for the insertion reaction described under "Experimental Procedures," except that the reticulocyte lysate translation mixture that was included did not contain [35S]methionine. The mixtures were chilled and incubated with the indicated amounts of proteinase K (PROT. K) for 15 min on ice. The proteolytic reaction was stopped with 2 mM PMSF, and the mitochondria were isolated by centrifugation, and reacted with 5 μM [3H]clorgyline before being analyzed by SDS-PAGE and fluorography. Panel B, portions of a translation mixture containing in vitro synthesized [35S]labeled human monoamine oxidase A were incubated at 37 °C for 30 min under the conditions for the insertion reaction described under "Experimental Procedures," except that mitochondria were omitted. The mixtures were chilled and incubated with the indicated amounts of proteinase K (PROT. K) for 15 min on ice. The proteolytic reaction was stopped with 2 mM PMSF, and the mitochondria were isolated and reacted for 60 min at 25 °C with 5 μM [3H]clorgyline before being analyzed by SDS-PAGE and fluorography. Panel C, 100 μg/ml rat liver mitochondria were incubated for 30 min at 37 °C under the conditions for the insertion reaction described under "Experimental Procedures" with translation mixture containing [35S]monoamine oxidase A. When indicated, ATP had been depleted from the reaction mixture. The mixtures were chilled and incubated with the indicated amounts of proteinase K for 15 min on ice. The proteolytic reaction was stopped with 2 mM PMSF, and the mitochondrial proteins were analyzed by SDS-PAGE and fluorography.
translation mixture containing in vitro synthesized \(^{35}S\)monoamine oxidase A (radiolabeled with \(^{35}S\)methionine during translation) was incubated with proteinase K under similar conditions but in the absence of mitochondria, the monoamine oxidase A was degraded by concentrations as low as 1 \(\mu g/ml\) and completely destroyed by 10 \(\mu g/ml\) proteinase (Fig. 1B). The omission of mitochondria only changed the concentration of protein in the incubation by 1–1.5%; this small change is unlikely to have affected the activity of proteinase K. These findings (and similar experiments performed with outer membranes isolated from rat liver mitochondria) indicated that protection from 10 \(\mu g/ml\) proteinase K could be used as an index of insertion of \(^{35}S\)monoamine oxidase A into rat liver mitochondrial outer membranes.

A aliquots of a translation mixture containing in vitro synthesized \(^{35}S\)monoamine oxidase A were incubated with rat liver mitochondria for 30 min at 37°C. Then the mitochondria were treated with proteinase K in concentrations up to 10 \(\mu g/ml\). The mitochondria were isolated by centrifugation and analyzed by SDS-PAGE and fluorography. As can be seen in Fig. 1C, \(^{35}S\)monoamine oxidase A was bound to the mitochondria. Term "bound" is used here in an operational sense to describe enzyme that is recovered in association with mitochondria but not necessarily properly inserted. When ATP and an ATP-regenerating system were present during the incubation, the mitochondrial \(^{35}S\)monoamine oxidase A was as resistant to proteinase K treatment as the endogenous enzyme (Fig. 1A). In some cases, ATP was depleted from the translation mixture containing \(^{35}S\)monoamine oxidase A by using apyrase. When these ATP-depleted translation mixtures were incubated with mitochondria in the absence of an ATP-generating system, the mitochondrially bound \(^{35}S\)monoamine oxidase A was virtually as sensitive to proteinase K as \(^{35}S\)monoamine oxidase A in the absence of mitochondria (Fig. 1B). These experiments indicated that monoamine oxidase A was able to bind to mitochondria in the absence of ATP, but that ATP was required for its insertion into the outer membranes and consequent protection from proteinase K.

As monoamine oxidase A is normally localized in the outer membranes of the mitochondria, it was important to ensure that \(^{35}S\)monoamine oxidase A was inserted into these membranes rather than imported by some artifactual process during the in vitro reaction. Translation mixtures containing \(^{35}S\)monoamine oxidase A were incubated with either mitochondria or isolated outer membrane in the presence of an ATP-regenerating system (Fig. 2). Neither the association of \(^{35}S\)monoamine oxidase A with the mitochondria nor the insertion (protection from proteinase K) of \(^{35}S\)monoamine oxidase A was inhibited if the mitochondrial membrane potential was discharged by prior incubation of the mitochondria with valinomycin. Import of mitochondrial precursors into the interior compartments of the organelle is well known to depend on a membrane potential (13), and it is, therefore, unlikely that \(^{35}S\)monoamine oxidase A was being artifically imported to the inner membrane, matrix, or intramembrane space. Finally, as has already been shown for monoamine oxidase B (16, 17), outer membranes isolated from rat liver mitochondria were also able to bind and insert \(^{35}S\)monoamine oxidase A, although to a lesser extent than intact mitochondria.

Previous work concerning the insertion of monoamine oxidase B into isolated outer membranes (1) demonstrated that both ATP and the peptide, ubiquitin, were required for this process. Since ubiquitin and the enzyme activities involved in its conjugation are known to be present in the reticulocyte lysate (30), experiments were performed to evaluate the importance of ubiquitin in \(^{35}S\)monoamine oxidase A insertion (Fig. 3). After \(^{35}S\)monoamine oxidase A synthesis was completed, it was possible to deplete ubiquitin from the translation mixture with IgGs that had been purified from antisera against ubiquitin. ATP was depleted by destroying the ATP in the translation mixture with apyrase and omitting the ATP-regenerating mixture from the incubations with mitochondria. As can be seen in Fig. 3A, depletion of either ATP or ubiquitin had no apparent effect on the binding of \(^{35}S\)monoamine oxidase A to mitochondria. On the other hand, depletion of either ATP or ubiquitin completely prevented the insertion of \(^{35}S\)monoamine oxidase A as judged by its sensitivity to proteinase K (Fig. 3B, lanes 2 and 4). Restoration of ATP or ubiquitin, respectively, to depleted reaction mixtures restored insertion of \(^{35}S\)monoamine oxidase A (Fig. 3B, lanes 3 and 5). It is unlikely that ubiquitin interfered with the proteinase K treatment of mitochondria after the insertion reaction, since addition of exogenous ubiquitin to an otherwise untreated reaction did not affect the insertion of \(^{35}S\)monoamine oxidase A (data not shown). Furthermore \(^{35}S\)monoamine oxidase A was completely destroyed by the proteinase if ATP but not ubiquitin was depleted from the translation mixture. It is also unlikely that the IgGs had an artifactual effect on the proteinase, since preimmune IgGs did not influence the insertion reaction. It seems clear that both ATP and ubiquitin are required for the insertion of \(^{35}S\)monoamine oxidase A.
Monoamine oxidase A into the mitochondrial outer membrane.

**The Reaction of in Vitro Synthesized Monoamine Oxidase A with Mechanism-based Inhibitors**—If the in vitro insertion of monoamine oxidase A mimics the corresponding in vivo process, the enzyme should acquire catalytic activity. The amounts of monoamine oxidase A synthesized in vitro were too small to measure by enzymatic activity. However, since the formation of a clorgyline adduct depends on the catalytic oxidation of this inhibitor by monoamine oxidase A, experiments were performed using [3H]clorgyline’s reaction with monoamine oxidase A to determine whether the in vitro synthesized monoamine oxidase A was catalytically active or was able to acquire catalytic activity by interaction with the outer membrane.

Monoamine oxidase A was synthesized in vitro with unlabeled methionine replacing the [35S]-labeled amino acid so that the resulting monoamine oxidase would not be radioactive. An aliquot of this “cold” translation mixture was reacted with 10 μM [3H]clorgyline and assayed by SDS-PAGE and fluorography for the presence of a tritium-labeled adduct. As can be seen in Fig. 4, lane 7, no such adduct was detected, indicating that the in vitro synthesized enzyme had little or no activity prior to its association with the outer membrane.

To determine whether monoamine oxidase acquired catalytic activity on interaction with the outer membranes, it was first necessary to inactivate the endogenous monoamine oxidase A activities. This was done by reacting the mitochondria with 1 mM of each of two irreversible monoamine oxidase inhibitors, clorgyline (monoamine oxidase A-selective) and pargyline (monoamine oxidase B-selective). The inhibitor-treated mitochondria were washed to remove unreacted monoamine oxidase A inhibitors and compared with untreated mitochondria for their ability to bind [3H]clorgyline. As can be seen from Fig. 4, lanes 1 and 2, the inhibitor-treated mitochondria were no longer able to react with the drug. These mitochondria were incubated, either in the presence of ATP or under conditions of ATP depletion (as indicated in Fig. 4), with monoamine oxidase A that had been synthesized in a “cold” translation mixture. The mitochondria were treated with proteinase K when isolated, reacted with 5 μM [3H]clorgyline, and analyzed by SDS-PAGE and fluorography. When the incubation was performed in the presence of ATP, a faint (compare with Fig. 4, lane 1) but clearly evident band was present in both intact and proteinase K-treated mitochondria (Fig. 4, lanes 3 and 4). When the reaction was performed after ATP depletion, similar evidence for adduct formation was seen in intact mitochondria but was not present after proteinase K treatment (Fig. 4, lanes 5 and 6). It was expected that small amounts of the [3H]clorgyline adduct would be found since only the limited amounts of active monoamine oxidase A that can be synthesized in vitro would be expected to be present in the mitochondria. The possibility that [3H]clorgyline was bound to a small fraction of the endogenous monoamine oxidase that was “reactivated” during the incubation is ruled out; endogenous monoamine oxidase was protected from proteinase K (Fig. 1A), whereas the [3H]clorgyline- monoamine oxidase A that was bound in the absence of ATP was destroyed without leaving any detectable proteinase K-resistant [3H]-labeled adduct (Fig. 4, lane 4). These data suggest that not only does monoamine oxidase insert into the outer membranes as an active enzyme, but that the inactive soluble “precursor” acquires catalytic activity on association with the outer membranes prior to the ATP-dependent insertion reaction.

**Mechanism-based Monoamine Oxidase Inhibitors Inhibit the Insertion of Monoamine Oxidase A**—The effects of two monoamine oxidase inhibitors, clorgyline and pargyline, were tested on the insertion of [35S]monoamine oxidase A. In some experiments, 10 μM clorgyline or pargyline were preincubated for 60 min at 25 °C with the complete reaction mixture except mitochondria before the insertion reaction was started (by the addition of the mitochondria). Neither of the monoamine oxidase inhibitors interfered with the ATP-dependent, proteinase K-resistant insertion of [35S]monoamine oxidase A (data not shown), indicating that clorgyline and pargyline were not able to inhibit this process by interacting with ubiquitin, ubiquitin-activating enzymes, or the catalytically inactive unbound form of [35S]monoamine oxidase A (Fig. 4). However, it seemed possible that the inhibitors might affect the insertion if they were reacted with an enzymatically active form of [35S] monoamine oxidase A under conditions that allow significant amounts of the catalytically generated adducts to form (Fig. 4) (31). In experiments designed to test this argument (Fig. 5), aliquots of a translation mixture containing [35S] monoamine oxidase A were incubated with mitochondria in the absence of ATP so that the [35S]monoamine oxidase A could associate with the membranes and become enzymatically active but not inserted. These mixtures were incubated with 0.1 to 10 μM clorgyline or pargyline. After incubation with the inhibitors, ATP was added; the incubation was continued and the insertion of [35S] monoamine oxidase A was evaluated. As can be seen from Fig. 5A, both drugs inhibited the ATP-dependent insertion reaction although clorgyline was somewhat more efficacious. The inhibitory effects of the two monoamine oxidase inhibitors on the insertion reaction were evaluated by densitometry, and they were compared with the effects of the two drugs on the endogenous monoamine oxidase A activity of rat liver mitochondria. It can be seen from Fig. 5B that clorgyline is a more efficacious inhibitor of enzymatic activity than of insertion, whereas pargyline is less selective (Fig. 5C). It is unlikely that the drugs interfered with insertion by affecting the mitochondria in some deleterious way, since treatment of mitochondria with much higher concentrations of both inhibitors did not

![Fig. 4. Monoamine oxidase A that is bound to or inserted into mitochondria in vitro can react with [3H]clorgyline. Mitochondria were treated with pargyline and clorgyline (each 1 mM), as described under "Experimental Procedures." 100-μg samples of the mitochondria were incubated for 60 min at 25 °C with 5 μM [3H]clorgyline before (lane 1) and after (lane 2) this treatment. These mitochondria were isolated and analyzed by SDS-PAGE and fluorography. 100 μg/ml inhibitor-treated mitochondria were incubated for 30 min at 37 °C under the conditions for the insertion reaction, as described under "Experimental Procedures," except that the in vitro synthesized monoamine oxidase A was not labeled with [35S]methionine (lanes 3-6). In one instance, mitochondria were omitted from the reaction (lane 7). ATP was depleted when indicated ("Experimental Procedures"). The reactions were treated with proteinase K and PMSF as described in the legend to Fig. 1 when indicated. Finally, the mitochondria were isolated, reacted for 60 min at 25 °C with 5 μM [3H]clorgyline, and analyzed by SDS-PAGE and fluorography. In the instance when mitochondria were omitted (lane 7), the entire reaction mixture was incubated with [3H]clorgyline and analyzed as above. [3H]CLORG. MITO, [3H]clorgyline-treated mitochondria; MAO INHIB. MITO, monoamine oxidase inhibitor-treated mitochondria; MAO A, monoamine oxidase A; [3H]CLORG, [3H]clorgyline; PROT. K, proteinase K.](image-url)
The data presented here suggest that monoamine oxidase A inserts into the outer membrane of mitochondria by a two-step process such as that depicted in Fig. 6. According to this model, monoamine oxidase A is released from polysomes as a soluble precursor. The enzyme is then able to bind to the outer membrane in an "insertion-competent" form. This binding does not require ATP; however, the subsequent insertion reaction requires not only ATP but the polypeptide, ubiquitin. The fact that both are required suggests that a ubiquitin-protein conjugate may be formed. Although there is, at present, no direct evidence for such a conjugate, we have previously reported that a mutant form of ubiquitin that does not undergo the ATP-dependent reaction with the ubiquitin-activating enzyme, E1, did not support the insertion of bovine monoamine oxidase B, whereas wild type ubiquitin did (17). In any case, our previous work and the experiments presented here indicate that ubiquitin plays some role in the insertion of both monoamine oxidase isoenzymes into the outer membrane.

It appears that, whereas monoamine oxidase A is catalytically inactive prior to its interaction with the outer membrane, the enzyme acquires activity upon its initial binding with the membrane. This conclusion is based on the observation that before its binding to the mitochondrial outer membrane, monoamine oxidase A was unable to catalyze its own alkylation by clorgyline; however, both the membrane-bound (i.e. proteinase-sensitive) and membrane-inserted (i.e. proteinase-resistant) forms were able to catalyze this alkylation. The finding that both clorgyline and pargyline (a less potent mechanism-based inhibitor of monoamine oxidase A) apparently reacted with membrane-bound monoamine oxidase A to prevent its insertion supports the conclusion that this form of the enzyme has catalytic activity. Since both the oxidation of these inhibitors and their subsequent adduct formation depends upon the presence of the covalently linked flavin (6), this cofactor must have been attached prior to the insertion of the monoamine oxidase A apoenzyme into the outer membrane.

prevent the insertion of monoamine oxidase A (Fig. 4). It is also unlikely that "cytosolic factors" were affected since, as was mentioned, 10 \( \mu \)M clorgyline or pargyline did not affect the insertion of the enzyme when they had been preincubated with all of the components of the reaction mixture except mitochondria. Considering that clorgyline has been shown to form an adduct with in vitro synthesized monoamine oxidase A after the enzyme's binding to the mitochondrial outer membranes (Fig. 4), it seems most probable that the impaired insertion is related to an interaction of the drugs with this form of monoamine oxidase A.
The dose-response curves for pargyline's inhibition of insertion (i.e., reaction with the membrane-associated human monoamine oxidase A) and substrate oxidation in mitochondria (i.e., reaction with the endogenous, membrane-inserted rat monoamine oxidase A) were so similar as to suggest that pargyline was an equally good substrate for both monoamine oxidase forms. On the other hand, the higher affinity inhibitor, clorgyline, was a better inhibitor of the catalysis performed by the membrane-inserted enzyme than of the insertion of the membrane-associated monoamine oxidase A. It is unlikely that the differential effect of clorgyline can be accounted for by the fact that the membrane-associated form is a human enzyme, whereas the membrane-inserted form is a rat enzyme, since human and rat monoamine oxidase A are equally sensitive to inhibition by clorgyline (32). It is more likely that the superficially bound and fully inserted enzymes have slightly different catalytic properties that are revealed by clorgyline but not pargyline. These properties cannot be identified from the experiments presented here; however, it is reasonable to surmise that they would be related to the active site of monoamine oxidase A. Zeller and co-workers (33) have proposed that monoamine oxidase A has three sites of interaction for substrates: an amine-binding site, a hydrophobic site, and a nucleophilic site. According to this model, substrates like clorgyline would be expected to bind only to the amine-binding and hydrophobic sites, whereas substrates like clorgyline would be expected to occupy all three. The differential inhibitory effects of clorgyline but not pargyline might be explained if the nucleophilic site did not form completely until the enzyme achieved its final conformation as a membrane-inserted protein.

The acquisition of catalytic activity by the membrane-bound monoamine oxidase indicates that the catalytic domain and, perhaps, other domains of this enzyme form have conformational similarity with the same domains of the membrane-inserted enzyme. At present, it is not clear whether these domains must undergo another conformational change during the ATP- and ubiquitin-dependent insertion reaction. In this connection, metathreaxate has been shown to inhibit the translocation of fusion proteins consisting of a mitochondrial presequence and mouse dihydrofolate reductase (34, 35). Methothreaxate is thought to interfere with conformational changes associated with the import of these proteins into mitochondria. It is possible that adduct formation by clorgyline and pargyline have similar effects on the insertion of monoamine oxidase A into mitochondrial outer membranes. That is, adduct formation may cause monoamine oxidase A to become refractory to conformational changes that may be required for its insertion.

Acknowledgments—We would like to acknowledge Dr. Creed Abell (University of Texas, Austin) and Dr. Vincent Chau (Wayne State University, Detroit) for their generous gifts of the cDNA for monoamine oxidase A and antisera against ubiquitin, respectively. We also thank Dr. Gottfried Schatz (Biezentrum, Basel) and Dr. Keith Vernier (Pennsylvania State University, Hershey) for reading this manuscript and for their helpful advice. Finally, we would like to thank Dr. Paul Hollenberg (Wayne State University, Detroit) for his support of this effort.

REFERENCES

1. Sottocasa, G. L., Kuylenstierna, B., Ernest, L., and Bergstrand, A. (1976) J. Cell Biol. 32, 415–438
2. Weiss, S., and McCauley, R. (1979) Proceedings of the Fourth International Catecholamine Symposium (Usdin, E., and Bar-chas, J., eds) pp. 198–202, Permaigon Press, New York
3. McCauley, R., and Racker, E. (1973) Mol. Cell. Biochem. 1, 73–86
4. Bach, A., Lan, N., Johnson, D., Abell, C., Benenek, M., Kwan, S.-W., Seeberg, P., and Shih, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4934–4938
5. Smith, D., Filipowicz, C., and McCauley, R. (1986) Biochem. Biophys. Acta 831, 1–7
6. Chuang, H., Petek, D., and Hellerman, L. (1974) J. Biol. Chem. 249, 2381–2384
7. Soliner, T., Griffiths, G., Pfaller, R., Pfanner, N., and Neupert, W. (1989) Cell 59, 1061–1070
8. Pfaller, R., and Neupert, W. (1987) EMBO J. 6, 2635–2642
9. Gasser, S. M., and Schatz, G. (1983) J. Biol. Chem. 258, 3427–3430
10. Hwang, S., and Schatz, G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5432–5436
11. Pfanner, N., Pfaller, R., Kleene, R., Ito, M., Troupchung, M., and Neupert, W. (1988) J. Biol. Chem. 263, 4049–4051
12. Chen, W.-J., and Douglas, M. (1987) Cell 49, 651–658
13. Verner, K., and Schatz, G. (1987) EMBO J. 6, 2449–2456
14. Shore, G., Power, F., Bendayam, M., and Carignan, P. (1981) J. Biol. Chem. 256, 8761–8766
15. Ono, H., and Tuboi, S. (1987) Eur. J. Biochem. 168, 509–514
16. Zhuang, Z., Hogan, M., and McCauley, R. (1988) FEBS Lett. 238, 185–190
17. Zhuang, Z., and McCauley, R. (1989) J. Biol. Chem. 264, 14594–14596
18. Finey, D., Bartel, B., and Varshavsky, A. (1989) Nature 338, 394–401
19. Finey, D., Ozkaynak, E., and Varshavsky, A. (1987) Cell 48, 1035–1046
20. Hershko, A. (1988) J. Biol. Chem. 263, 15237–15240
21. Martinez, P., and McCauley, R. (1977) Biochim. Biophys. Acta 497, 437–446
22. Schenborn, E., and Mierendorf, R. (1985) Nucleic Acids Res. 12, 7035–7056
23. Melton, D., Krieg, P., Rebagliati, M., Maniatis, T., Zinn, K., and Green, M. (1984) Nucleic Acids Res. 12, 7035–7056
24. Pelham, H., and Jackson, R. (1976) Eur. J. Biochem. 67, 247–256
25. Meyer, E., West, C., and Chau, V. (1986) J. Biol. Chem. 261, 14365–14368
26. Goding, J. (1978) J. Immunol. Methods 20, 241–253
27. Cantillo, J. (1984) J. N-Alkylation of Sulfoximines. ZI. Synthesis of Sulfonyl Analog of Procainamide and Clorgyline. Doctoral dissertation, Wayne State University
28. Navarro-Welch, C., and McCarley, R. (1982) J. Biol. Chem. 257, 13645–13649
29. Lowry, O., Rosebrough, N., Farr, A., and Randall, R. (1951) J. Biol. Chem. 193, 265–275
30. Rechsteiner, M. (1987) Annu. Rev. Cell Biol. 3, 1–30
31. McCauley, R. (1976) Biochem. Pharmacol. 25, 2214–2215
32. Sullivan, J., McDornell, L., Hardiman, O., Farrell, M., Phillips, J., and Tipton, K. (1986) Biochem. Pharmacol. 35, 3255–3260
33. Zeller, E., Arora, K., Gurney, D., and Huprikar, S. (1976) in Monoamine Oxidase: Structure, Function and Altered Functions, (Singer, T., Von Korff, R., and Murphy, D., eds) pp. 101–120, Academic Press, San Diego
34. Eilers, M., and Schatz, G. (1986) Nature 322, 228–232
35. Rassow, J., Cuiard, B., Weinzihue, U., Herzog, V., Hartl, P.-U., and Neupert, W. (1989) J. Cell Biol. 109, 1421–1428