Biochemical Pharmacology, Vol. 33, No. 24, pp. 3929–3932, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

PURIFICATION OF ENDOGENOUS MODULATORS OF MONOAMINE OXIDASE FROM PLASMA

CECILIA T. GIAMBALVO

Rhode Island Psychiatric Research and Training Center, Cranston, RI; Neuroscience Department of Brown University, Providence, RI; and Department of Pharmacology, University of Rhode Island, Kingston, RI, U.S.A.

(*Received* 1 *September* 1983; *accepted* 12 *January* 1984)

Abstract—A partial purification of endogenous modulators of monoamine oxidase-A (MAO-A) and MAO-B from human plasma has been achieved through Sephadex, ion-exchange and affinity chromatography. The MAO-A modulator had a molecular weight of about 4,000, was acidic or neutral, and did not contain the carbohydrate moiety mannose. It was thermostable but sensitive to trypsin treatment. It inhibited MAO-A activity in a sonically disrupted mitochondrial preparation prepared from bovine striatum in a dose-dependent manner and altered the kinetic parameters of MAO-A by increasing the K_m and decreasing the V_{max}. The concentration of the MAO-A modulator was higher than that of the MAO-B modulators, which had molecular weights of about 7,000, 14,000 and >30,000 respectively. The MAO-B modulators increased the K_m for tryptamine without changing the V_{max}. These data indicate that human plasma contains peptides that may function as endogenous regulations of platelet MAO. The significance of this finding in relation to psychiatric disorders is discussed.

Monoamine oxidase (EC 1.4.3.4, amine:O_2 oxidoreductase, MAO) catalyzes the oxidative deamination of a variety of monoamines to their corresponding aldehydes, which are further metabolized by aldehyde dehydrogenases and reductases to the acid and alcohol metabolites. MAO activity has been subdivided on a pharmacological basis into two types: MAO-A and MAO-B, where the A form is sensitive to low concentrations of clorgyline and the B form to low concentrations of l-deprenyl [2]. Both forms are present in the brain, with 5-hydropytryptamine (5-HT) being metabolized predominantly by MAO-A, and benzylamine and low concentrations of β-phenylethylamine by MAO-B; tyramine and tryptamine are metabolized by both forms [3–6]. In human platelets, only the B form of the enzyme is present [7]. There is some evidence to indicate that MAO-A and B may be two distinct mitochondrial proteins with different molecular weights [8, 9]. This multiple protein concept has been challenged by an alternative view that MAO may be a single enzyme with multiple binding sites that can be differentially affected by changes in the membrane phospholipid environment [10–13]. Indeed, immunochemical characterization of MAO-A and B purified from placenta and platelets indicate that they have many antigenic properties in common. However, the finding that a monoclonal antibody to platelets can immunoprecipitate MAO-B but not MAO-A suggests that they may be structurally different polypeptides [14].

Recent studies showing that the addition of plasma or urine *in vitro* can activate [15] or inhibit [16–18] MAO activity have led to the suggestion that MAO may be regulated by endogenous modulators. We have also recently demonstrated that the addition of plasma to platelets inhibits MAO-A and B activities as measured by the deamination of 5-HT by bovine striatum enzyme and of tryptamine by platelet enzyme [18]. These inhibitors seem to be small molecular weight proteins: the inhibition of MAO persisted after filtration of the plasma through Amicon PM30 membranes to remove substances > 30,000 mol. wt, but was abolished by prior precipitation of plasma proteins with perchloric acid [18]. To further characterize these inhibitors, we have fractionated plasma through liquid chromatography and then examined the biochemical properties of these partially purified inhibitors. We now report that these inhibitors are thermostable, trypsin-sensitive, small molecular weight proteins.

MATERIALS AND METHODS

Human platelet-poor plasma (about 40 ml) was obtained from the blood bank and filtered through Amicon PM30 membranes to remove substances > 30,00 mol. wt [18]. The filtrate was lyophilized, redissolved in 2 ml of water, and loaded onto a Sephadex G-50 column (1.5 × 50 cm). The column had been calibrated previously with the known molecular weight markers glucagon, ribonuclease, trypsin, pepsin and bovine serum albumin. The column was eluted with 50 mM potassium phosphate buffer containing 0.1 M KCl, pH 7.4 ("column buffer"). Fractions (3 ml) were collected and monitored for protein concentration at 280 nm in a spectrophotometer using bovine serum albumin as standard. Fractions (400 μl) were added to sonically disrupted mitochondrial fractions to determine their effects on both MAO-A and B activities. MAO-A

activity was measured by the deamination of [^{14}C]-HT (50–500 μM) in bovine striatal tissues, while MAO-B activity was measured by the deamination of [^{14}C]tryptamine (5–50 μM) in human platelets as described previously [18]. Control and blanks contained an equal volume of the column buffer to maintain a constant salt concentration. The column blanks did not contain any MAO inhibitory activity. The concentration of inhibitor is expressed as units of inhibition per ml of plasma and specific activity as units of inhibition per mg protein, where 1 unit represents 1% inhibition of MAO activity.

Further purification of the inhibitor was carried out by ion-exchange chromatography. An aliquot (1 ml) of the peak inhibitory fraction isolated from the Sephadex column was loaded onto a Dowex 50 × 8 (H^+) column (0.6 × 10 cm) (220–400 mesh). The column was eluted with 10 ml of water followed by 10 ml of 2 N ammonium hydroxide. Fractions of 1 ml each were collected and added to mitochondrial preparations to determine their effects on MAO activity. Since ammonia itself inhibited MAO activity, the ammonia eluates were evaporated to dryness under nitrogen and redissolved in water prior to the addition to the mitochondrial fractions for assay of MAO activity.

The modulator was also purified by affinity chromatography. An aliquot (1 ml) of the peak inhibitory fraction eluted off the Sephadex column was loaded onto an agarose-concanavalin-A column. The column was eluted with 2 ml of buffer followed by 10 ml of 0.3 M α-methyl-mannoside. Fractions (1 ml) were collected and added to mitochondrial preparations to determine their effects on MAO activity.

For determination of the heat stability of the modulator, an aliquot of the Sephadex eluate containing peak inhibitory activity was headed at 95° for 5 min prior to its addition to the mitochondrial preparations. To determine if the modulator is a protein that is susceptible to trypsin treatment, the Sephadex eluate was incubated with a 5 mg/ml trypsin solution at 37° for 1 hr. The trypsin was then inactivated by heating at 95° for 5 min (since it was found that the modulator was thermostable while the trypsin was not). The heated sample was then added to mitochondrial preparations to determine their effects on MAO activity. Controls containing an equal volume of the buffer instead of the modulator were treated with trypsin and then heated in the same manner. These controls showed a slight inhibition of MAO activity (11%) compared to controls containing no trypsin.

RESULTS

A typical elution profile of plasma from the Sephadex column is shown in Figs. 1 and 2. When the eluted fractions were added to sonicated striatal tissues using [^{14}C]5-HT as substrate, one inhibitory peak against MAO-A was found (Fig. 1), corresponding to a molecular weight of 4,000. The V_e/V_o for the markets are as follows: glucagon, (mol. wt 3500) 2.31; ribonuclease, (mol. wt 13,700) 1.53; trypsin (mol. wt 24,000) 1.36; and BSA, (mol. wt 67,000) 1.00. When the eluted fractions were added

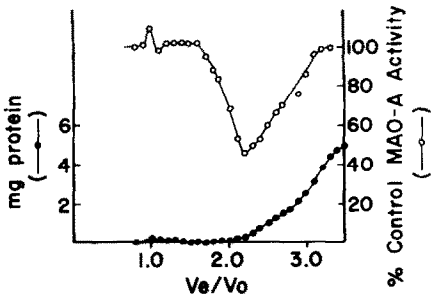

Fig. 1. Elution profile of the MAO-A modulator from the Sephadex column. The total mg of protein in each 3-ml fraction (280 nm) (——●——) and the effects of each 400-μl aliquot on MAO activity (as expressed in percent of control activity) (——○——) were determined as described in the text.

to platelets using [^{14}C]tryptamine as substrate, a different profile was observed: there were three inhibitory peaks to MAO-B, corresponding to molecular weights of >30,000, 14,000 and 7,000 respectively (Fig. 2). The specific activities of the inhibitors were computed from three experiments (Table 1): the specific activity of the MAO-A inhibitor was 298 ± 2 units/mg protein; the specific activities of the MAO-B inhibitors were 348 ± 65, 703 ± 24 and 343 ± 34 respectively.

The 4,000 molecular weight peak was then loaded onto a Dowex 50 column. All the inhibitory activity was recovered when the column was eluted with water (Fig. 3), indicating that the modulator is acidic or neutral in nature at the pH (7.4) of application to the Dowex column. The combined Sephadex and Dowex chromatography yielded a purification of several hundred fold (Table 1).

The 4,000 molecular weight fraction from the Sephadex column was found to inhibit MAO-A activity in a non-linear dose-dependent manner (Fig. 4). By using a double-reciprocal plot, the K_i was

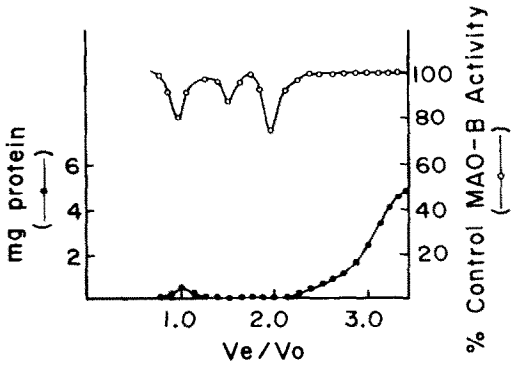

Fig. 2. Elution profile of the MAO-B modulators from the Sephadex column. The total mg of protein in each 3-ml fraction (280 nm) (——●——) and the effects of each 400-μl aliquot on MAO-B activity (expressed as percent of control activity (——○——) were determined as described in the text.

Table 1. Purification of the MAO modulators from human plasma*

	Sample	Total units	mg protein	Specific activity	Fold purification
I. MAO-A	i. Platelet-poor plasma	3200	2250	1.4	1
	ii. PM30 filtrate	3105	45	69	49
	iii. Sephadex eluate	3375	12	281†	201
	iv. Dowex 50 eluate	2423	1.8	1346	961
II. MAO-B	i. Crude extract	1822	2250	0.8	1
	ii. PM30 filtrate	578	45	13	16
	iii. Sephadex eluate I	135	0.4	337‡	422
	II	90	0.1	900‡	1125
	III	135	0.4	337‡	422

* Platelet-poor plasma (40 ml) was filtered through Amicon PM30 membranes and the filtrate was then lyophilized, redissolved in 2 ml of water, and loaded onto a Sephadex G-50 column. An aliquot containing the peak inhibitory activity was also loaded onto a Dowex 50 column. Aliquots of the eluates were assayed for protein concentration and MAO-A and MAO-B activities as described in the text. Total units present the sum of inhibitory activity under the peak derived from 40 ml of plasma, while specific activity is units of inhibition per mg protein. Sephadex eluate I, II and III are inhibitory peaks at V_e/V_o of 1.0, 1.6 and 1.9 respectively.

† Average specific activity of three column determinations was 298 ± 2.

‡ Average specific activities of three column determinations were 348 ± 65, 703 ± 24 and 343 ± 34 for V_e/V_o of 1.0, 1.6 and 1.9 respectively.

estimated to about 4×10^{-6} M. This assumed that all the protein in the fraction was due to the modulator, which had a molecular weight of 4,000. The modulator demonstrated the following properties: (1) an alteration in the kinetic parameters of MAO-A by increasing the K_m for its substrate and decreasing the V_{max} (Table 2); (2) susceptibility to trypsin treatment: it inhibited MAO-A activity by 17 and 18% after the trypsin treatment compared to 51 and 52% after treatment with inactivated trypsin (N = two experiments); (3) thermostability: heating at 95° for 5 min did not affect the inhibitory activity (44 and 52% inhibition for two experiments before heating vs 42 and 46% inhibition for two experiments after heating), and (4) apparent lack of the carbohydrate moiety mannose since it was eluted off the agarose-concanavalin column with buffer (Fig. 5) with >90% of activity recovered.

The MAO-B modulators eluted off the Sephadex column were present in a lower concentration than the MAO-A modulator. As shown in Table 1, the total number of units of MAO-A inhibitory activity recovered from the Sephadex column was 3,375 as compared to 135, 90 and 135 units of MAO-B inhibitory activity for fractions, I, II and III respectively. The MAO-B modulators altered the kinetic parameters of MAO-B by increasing the K_m for tryptamine without changing the V_{max} (Table 2).

DISCUSSION

We have reported recently [18] that addition of plasma to sonically disrupted mitochondrial preparations inhibits MAO-A and B activities *in vitro*. Based on the observations that the inhibition persisted after Amicon PM 30 filtration but was abolished by perchloric acid pretreatment, we suggested that the inhibitors may be small molecular weight proteins. This is supported by our present findings: the inhibitors were sensitive to trypsin treatment, and the Sephadex elution profile indicated that the MAO-A inhibitor was about 4,000 mol. wt and the MAO-B inhibitors about 7,000 and 14,000 and >30,000 mol. wt respectively. The difference in the molecular weights between the MAO-A and B inhibitors makes it likely that they are different proteins.

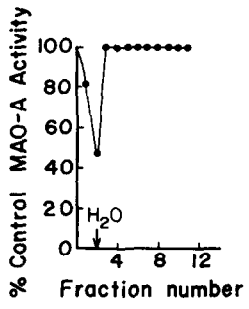

Fig. 3. Elution of the MAO-A modulator from the Dowex 50 column. The column was eluted with H_2O, followed by 2 N NH_4OH. Fractions (1 ml) were collected and assayed for their effects on MAO-A activity as described in the text.

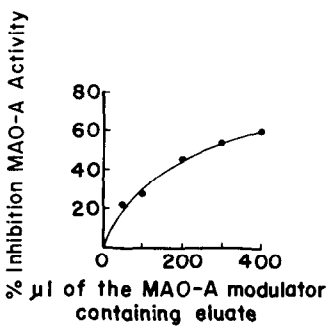

Fig. 4. Effects of increasing amounts of the Sephadex eluate containing the MAO-A modulator on MAO-A activity, which was measured with bovine striatal enzyme at 300 µM 5-HT.

Table 2. Effects of the MAO modulators on the kinetic parameters of MAO*

Type of MAO	Fraction (V_e/V_o)	% Control K_m	% Control V_{max}	N
MOA-A	2.2	188 ± 20	62 ± 3	3
MAO-B	1.0	119 ± 3	97 ± 1	3
	1.6	127 ± 5	104 ± 2	3
	1.9	119 ± 2	96 ± 3	3

* Aliquots of the Sephadex eluates with V_e/V_o ratios indicated above were assayed for their effect on the kinetic parameters of MAO-A and MAO-B as described in the text. Results are expressed as percent control K_m and V_{max} ± S.E.M. N is the number of determinations. Control MAO-A had a K_m of 186 ± 22 μM and a V_{max} of 37.3 ± 1.6 nmoles/hr/mg protein. Control MAO-B had a K_m of 14.0 ± 1.6 μM and a V_{max} of 49.0 ± 2.0 nmoles/hr/mg protein.

The MAO-A inhibitor also had the following properties: it was thermostable to heating at 95° for 5 min, although its stability at other times and temperatures has not yet been determined; it is not a mannose-containing glycoprotein since it did not bind to the agarose-concanavalin-A column; it inhibited MAO-A with an approximate K_i of 4×10^{-6} M; the inhibition was due to an increase in the K_m and a decrease in the V_{max}. From these results, it can be concluded that human plasma contains small molecular weight proteins that can inhibit MAO *in vitro*.

Our results also indicate that plasma contains a higher concentration of the MAO-A inhibitor than the MAO-B inhibitor. This was unexpected since platelets contain only MAO-B. It is, however, analogous to the finding that platelets have a high affinity uptake system for 5-HT even though they have a low ability to deaminate 5-HT [19]. Conversely, 5-HT neurons in the dorsal raphe nuclei were found to stain immunocytochemically for MAO-B even though 5-HT has a low affinity for MAO-B as compared to MAO-A [20].

The relationship between the plasma inhibitors and platelet MAO activity has yet to be determined. This information is relevant to studies linking low platelet MAO activity with alcoholism, schizophrenia and depressive disorders [21]. Low platelet MAO activity has also been proposed as a marker for vulnerability to psychiatric illness [22]. One possibility is that the low platelet MAO activity in these disorders may be accounted for by an increase in the level of the endogenous MAO inhibitors. A possible link to psychiatric disorders is further strengthened by our recent finding of similar inhibitory proteins to MAO in human CSF, indicating that these inhibitors are also produced in the CNS in addition to the periphery [23].

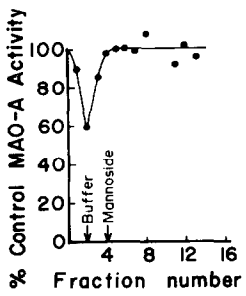

Fig. 5. Elution profile of the MAO-A modulator from the agarose-concanavalin-A column. The column was eluted with 2 ml of buffer, followed by 10 ml of α-methyl-mannoside. Fractions (1 ml) were collected and assayed for their effects on MAO-A activity as described in the text.

Acknowledgements—The author gratefully acknowledges the generous encouragement and moral support of Dr. R. E. Becker and to Dr. Robert Patrick for reviewing the manuscript. This work was being funded by the Institute of Mental Health, a division of Rhode Island Mental Health, Retardation and Hospitals.

REFERENCES

1. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
2. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 393 (1972).
3. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
4. J. A. Roth, *J. Neurochem.* **27**, 1107 (1976).
5. K. F. Tipton, M. D. Houslay and N. J. Garrett, *Nature New Biol.* **246**, 213 (1973).
6. H. L. White and A. T. Glassman, *J. Neurochem.* **29**, 987 (1977).
7. C. H. Donnelly and D. L. Murphy, *Biochem. Pharmac.* **26**, 853 (1977).
8. C. K. Brown, J. F. Powell and I. W. Craig, *Biochem. Pharmac.* **29**, 2595 (1980).
9. R. M. Cawthon, J. E. Pintar, F. P. Haseltine and X. O. Breakefield, *J. Neurochem.* **37**, 363 (1981).
10. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19**, 467 (1976).
11. M. Jain, *Life Sci.* **20**, 1925 (1977).
12. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
13. A. Schurr, *Life Sci.* **30**, 1059 (1982).
14. R. M. Denney, N. T. Patel, R. R. Fritz and C. W. Abell, *Molec. Pharmac.* **22**, 500 (1982).
15. P. H. Yu and A. A. Boulton, *Life Sci.* **25**, 31 (1979).
16. W. H. Berrettini and W. H. Vogel, *Am. J. Psychiat.* **135**, 605 (1978).
17. V. Glover, S. K. Bhattacharya and M. Sandler, *Nature, Lond.* **292**, 347 (1981).
18. C. T. Giambalvo and R. E. Becker, *Life Sci.* **29**, 2010 (1981).
19. S. M. Stahl and H. Y. Meltzer, *Expl. Neurol.* **59**, 1 (1978).
20. P. Levitt, J. E. Pintar and X. O. Breakefield, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6385 (1982).
21. C. J. Fowler, K. F. Tipton, A. V. P. Mackay and M. B. H. Youdim, *Neuroscience* **7**, 1577 (1982).
22. M. S. Buchsbaum, R. D. Coursey and D. L. Murphy, *Science* **194**, 339 (1976).
23. R. E. Becker, C. T. Giambalvo, R. A. Fox and M. Macho, *Science* **221**, 476 (1983).