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HUMAN BLOOD PLATELET MONOAMINE OXIDASE

G. G. S. COLLINS and M. SANDLER

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics & Gynaecology, Queen Charlotte's Maternity Hospital, London, W.6

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Abstract—Human blood platelet monoamine oxidase (MAO; EC 1.4.3.4) has been solubilized and purified about 12-fold. In contrast with MAO from other human tissues previously investigated, the enzyme is electrophoretically homogeneous and has a molecular weight of 235,000. Its substrate specificities are similar to those of plasma benzylamine oxidase but its lack of sensitivity to isoniazid and semicarbazide distinguishes it from the plasma enzyme. The differences between the physicochemical properties of platelet MAO and isoenzymes from other anatomical sites suggests that the platelet enzyme is unlikely to provide a satisfactory index of *in vivo* activity in the whole organism.

IT SEEMS likely that fluctuations in monoamine oxidase (monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4) (MAO) activity occur in man in response to certain physiological (menstrual cycle,[1] diet,[2] gut flora[3]) and pathological (toxaemia of pregnancy,[4] thyrotoxicosis,[5] megaloblastic anaemia[6]) events. Profound decreases in activity are commonly induced in clinical practice by the administration of MAO-inhibiting drugs[7] yet relatively few attempts to quantify these alterations have been reported in the literature. The reason is simple; although many different *in vivo* assay procedures have been devised, none is completely satisfactory.

Whilst indirect procedures have been employed, involving the measurement of urinary tryptamine excretion[8] or of 5-hydroxyindoleacetic acid excretion after an oral 5-hydroxytryptamine load,[9] they may give rise to conflicting data.[10] Direct measurement of the enzyme activity of buccal scrapings[11] has given variable results in this laboratory,* whilst obtaining jejunal biopsy specimens for assay[12] is not without risk to the patient. However, relatively high MAO activity is normally present in blood platelets[7,13,14] and such measurements have recently been employed as a measure of the status of MAO in other tissues of the patient.[14,15] Robinson *et al.*[14] justified such an extrapolation by claiming that both human platelet and liver MAO possess similar substrate and inhibitor specificities.

It has recently been reported that MAO may exist in multiple forms depending on tissue and species.[16–24] It therefore seemed of interest to purify and characterize human blood platelet MAO and by comparing its physico–chemical properties with those of MAO isoenzymes from other anatomical sites, particularly brain and liver, to investigate whether the assumption that platelet MAO activity reflects that of other tissues is warranted.

* J. Southgate, unpublished observations.

MATERIALS AND METHODS

Fresh blood was collected into 1 pint plastic bags containing 75 ml of acid–citrate–dextrose solution (ACD) (dihydric tri-sodium citrate, 1·54 g; monohydric citric acid, 0·55 g; anhydrous dextrose, 3·00 g; water to 120 ml). The bag was centrifuged at 275 *g* for 30 min to sediment red cells and leucocytes. The platelet-rich plasma was transferred to another bag containing a further 30 ml of ACD and spun at 1300 *g* for 30 min. The platelet residue was re-suspended in 30 ml of physiological saline, spun at 1300 *g* for 30 min and the platelets retained. By this method, the platelets from 7 pints of blood were harvested. All operations were carried out at 0°.

The platelets were suspended in ice-cold 0·3 M sucrose, mitochondria isolated as described by Hawkins[25] and MAO solubilized using sonic oscillations in the presence of benzylamine (10^{-3} M) and 1·0% v/v of the non-ionic detergent "Triton X-100".[19] The enzyme was purified by ammonium sulphate fractionation, the precipitate separating at 30–55 per cent saturation isolated by centrifugation (12,500 *g*, 20 min, 4°), dissolved in a minimum of phosphate buffer (0·05 M, pH 7·4) and further purified by gel-filtration through a 15 × 900 mm column of Sephadex G-200.

Enzyme activity was estimated (37°, pH 7·4) spectrophotofluorimetrically with kynuramine as substrate[26] and radiochemically[14] using ^{14}C-dopamine, ^{14}C-benzylamine, ^{14}C-tryptamine or ^{14}C-tyramine as substrates. The incubation mixtures contained 0·1 ml of labelled substrate (approximately 50,000 counts/min and 0·5 ml of the enzyme preparation in phosphate buffer (0·05 M, pH 7·4). After incubation for 30 min, the radioactive products were isolated by ion exchange chromatography[14] and counted in a Packard Liquid Scintillation Spectrometer (model 3320). The counting efficiency of each sample was calculated using the channels ratio procedure devised by Baillie[27] and was usually about 35 per cent. Protein concentrations were measured by the method of Lowry *et al.*[28] using bovine serum albumin as standard. Enzyme activity has been expressed in units, one unit being that amount of MAO which deaminates one mμ M of substrate/min at 37° and a pH value of 7·4.

Finally, polyacrylamide gel electrophoresis was carried out on the purified, solubilized enzyme preparations[18] and the band of MAO protein localized by the histochemical procedure described by Glenner *et al.*[29] using tryptamine as substrate. Protein bands were extracted from the gels[17] and their enzyme activity measured.

Chemicals. All chemicals were of the highest purity obtainable. 2-^{14}C-tryptamine HCl (3·6 mc/mM) was obtained from New England Nuclear Corporation, Boston Mass., U.S.A.; 7-^{14}C-benzylamine (10 mc/mM) from International Chemical Nuclear Corporation, City of Industry, Calif., U.S.A.; 1-^{14}C-tyramine HCl (43·7 mc/mM) and 2-^{14}C-dopamine (40 mc/mM) from the Radiochemical Centre, Amersham, England; M & B 9302 (clorgyline) was kindly donated by May and Baker Ltd.

RESULTS

Purification of human platelet MAO. The purification stages of human platelet MAO are listed in Table 1; after passage of the solubilized enzyme through a Sephadex G-200 column, there was a 12-fold final purification over the starting material. The molecular weight of the enzyme was estimated by gel filtration[30] using horse heart cytochrome c, bovine serum albumin, bovine γ-globulin and bovine thyroglobulin

as standards. A single sharp peak of enzyme activity coincided with the protein peak (Fig. 1) and corresponded to a molecular weight of 235,000 (mean of two experiments).

Substrate specificity of platelet MAO. Table 2 shows the activity of platelet MAO towards dopamine, tryptamine, tyramine, kynuramine and benzylamine at various stages in its purification. The rate of oxidation of dopamine was only about one twentieth that of benzylamine, the most actively metabolized substrate investigated. The ratio of specific activities towards the different substrates remained relatively constant throughout the purification procedure. The corresponding Michaelis

TABLE 1. PURIFICATION OF HUMAN PLATELET MAO

Purification step	Volume (ml)	Total protein (mg)	Total activity (units)	Activity (units/ml)	Activity (units/mg)	Yield (%)	Purification
Lysed whole platelets	100	1875	424	4·24	0·226	100	0
Isolated mitochondria	100	875	229	2·29	0·262	54·0	1·2
Sonicate 1 hr	104	360	162	1·56	0·450	38·2	2·0
2 hr	103	348	167	1·52	0·480	39·4	2·1
Ammonium sulphate (30–55% saturation)	6·8	91·8	46·2	5·49	0·503	10·9	2·2
Sephadex G-200	12·4	16·6	44·7	3·61	2·70	10·5	12·0
Gel-electrophoresis (of ammonium sulphate precipitate)	—	—	—	—	1·64	—	7·3
Gel-electrophoresis (of Sephadex G-200 eluate)	—	—	—	—	2·40	—	10·6

1 Unit of activity is defined as that amount of enzyme which deaminates 1 mμ M of substrate per minute at 37° and pH 7·4.

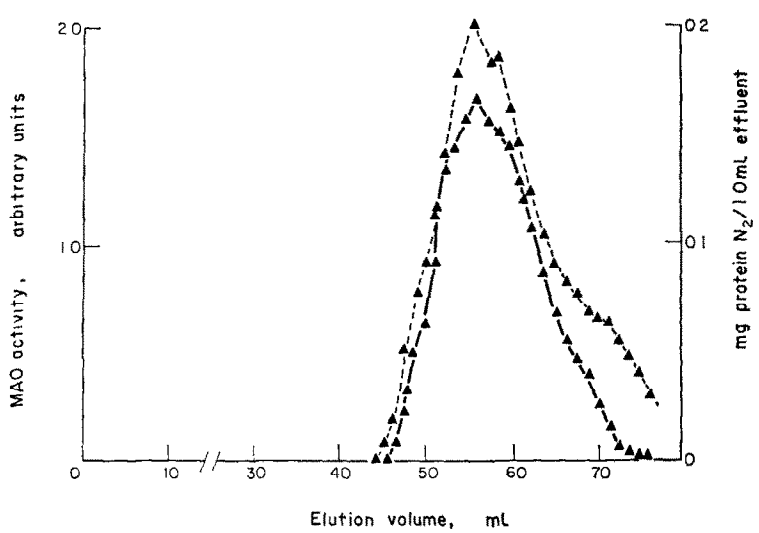

FIG. 1. Purification of soluble human blood platelet MAO through Sephadex G-200. Maximal enzyme activity and peak protein concentration both occur at an elution volume of 56·2 ml, equivalent to a molecular weight of 235,000.[30]

TABLE 2. THE SUBSTRATE SPECIFICITY OF HUMAN PLATELET MAO AT VARIOUS STAGES DURING ITS PURIFICATION (SEE TABLE 1)

Purification step	Substrate				
	Dopamine	Tryptamine	Tyramine	Kynuramine	Benzylamine
Lysed platelets	0·052 ± 0·010 (1	0·084 ± 0·005 1·62	0·244 ± 0·026 4·69	0·249 ± 0·035 4·79	0·947 ± 0·094 18·2)
Sonicate (2 hr)	0·109 ± 0·011 (1	0·172 ± 0·021 1·58	0·473 ± 0·062 4·34	0·472 ± 0·051 4·33	1·92 ± 0·22 17·6)
Electrophoresis	0·305 ± 0·027 (1	0·516 ± 0·062 1·69	1·37 ± 0·15 4·50	1·31 ± 0·11 4·28	5·77 ± 0·63 18·9)

The figures represent units of enzyme activity/mg protein ± S.E. of the mean. The figures in parentheses represent the ratios of the different activities.

TABLE 3. MICHAELIS CONSTANTS (K_m VALUES) AND V_{max} VALUES OF PURIFIED PLATELET MAO

Substrate	K_m	V_{max}
Benzylamine	$5{\cdot}50 \times 10^{-5}$	100
Tyramine	$3{\cdot}11 \times 10^{-5}$	24·6
Kynuramine	$3{\cdot}85 \times 10^{-5}$	27·2
Tryptamine	$7{\cdot}99 \times 10^{-6}$	15·4
Dopamine	$1{\cdot}92 \times 10^{-5}$	10·3

K_m and V_{max} values were derived from double reciprocal plots; V_{max} values are expressed as percentages of that obtained using benzylamine as substrate.

constants (K_m values) are shown in Table 3 together with V_{max} values expressed as percentages of the maximal rate of benzylamine oxidation. The affinity of the enzyme was almost seven times greater for tryptamine than for benzylamine whereas that for dopamine was of an intermediate value.

Inhibition of platelet MAO. Inhibitor constants (K_i values) for harmaline, iproniazid, pargyline, pheniprazine and clorgyline using kynuramine and tryptamine as substrates were estimated from double reciprocal plots and are shown in Table 4.

TABLE 4. K_i VALUES FOR PURIFIED HUMAN PLATELET MAO FOR VARIOUS INHIBITORS USING KYNURAMINE AND TRYPTAMINE AS SUBSTRATES

Inhibitor	Kynuramine	Tryptamine
Harmaline	$3{\cdot}9 \times 10^{-5}$ M	$8{\cdot}9 \times 10^{-5}$ M
Iproniazid	$2{\cdot}4 \times 10^{-6}$ M	$6{\cdot}8 \times 10^{-6}$ M
Pargyline	$1{\cdot}6 \times 10^{-8}$ M	$1{\cdot}3 \times 10^{-8}$ M
Pheniprazine	$1{\cdot}4 \times 10^{-7}$ M	$2{\cdot}2 \times 10^{-7}$ M
Clorgyline	$4{\cdot}5 \times 10^{-7}$ M	$5{\cdot}4 \times 10^{-7}$ M

Each inhibitor was incubated with enzyme for 30 min prior to addition of substrate. K_i values were calculated from double reciprocal plots.

Harmaline, a fully reversible inhibitor was least effective whereas the irreversible inhibitor pargyline was more than 2000 times as active. K_i values obtained using either kynuramine or tryptamine as substrates were similar for pargyline, pheniprazine and clorgyline but for harmaline and iproniazid the concentrations required in the presence of tryptamine were more than doubled. Neither semicarbazide nor isoniazid caused a greater than 10 per cent inhibition of enzyme activity even at a concentration of 10^{-3} M.

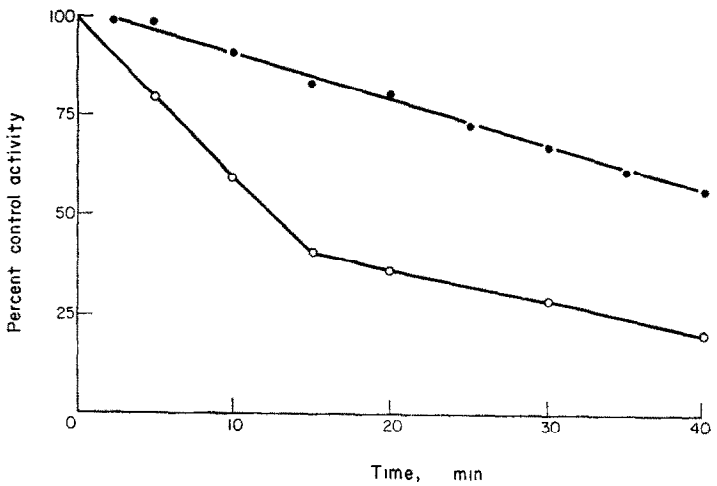

FIG. 2. Effect of temperature on MAO activity of whole lysed platelets (●—●) and purified platelet MAO (○—○). Enzyme preparations were suspended in phosphate buffer (0·05 M, pH 7·4) and heated to 50° for varying times. The results are expressed as percentages of control activity.

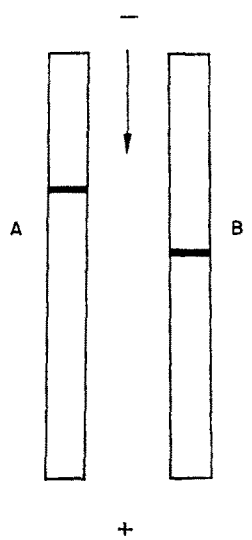

FIG. 3. Polyacrylamide gel electrophoresis of soluble human platelet MAO. Enzyme preparation (200 μl) was mixed with Sephadex G-200 and introduced on to the gel. Electrophoresis was continued at room temperature for 2 hr at 6 mA per tube using tris-HCl buffer (0·05 M) at pH 8·6 (A) and at pH 9·1 (B). Enzyme activity was demonstrated in the gels by the histochemical procedure of Glenner *et al.*[29] No activity was found at the origin or migrated toward the cathode.

The heat stability of human platelet MAO. When either lysed platelets or purified platelet MAO (0·5 units/ml) was suspended in phosphate buffer (0·05 M, pH 7·4) and heated to 50°, there was a loss of enzymic activity (Fig. 2). The fall in activity of the whole platelet preparation was linear with time whereas that of the purified enzyme appeared biphasic. In addition, the purified protein was more heat labile than the whole platelet preparation.

Polyacrylamide gel electrophoresis of human platelet MAO. Polyacrylamide gel electrophoresis of human platelet MAO in a continuous buffer system of 0·05 M tris-HCl at either pH 8·6 or 9·1 resulted in a single band of activity entering the gel from cathode to anode. No residual activity remained at the origin (Fig. 3) and no activity migrated from anode to cathode.

DISCUSSION

The combined use of ultrasonic disintegration, ammonium sulphate fractionation, gel filtration and polyacrylamide gel electrophoresis has enabled blood platelet MAO to be purified about 12-fold. The resulting soluble enzyme preparation was homogeneous with regard to molecular weight and showed only one band of activity on electrophoresis using two different buffer systems. As the relative activity of the enzyme towards dopamine, tryptamine, tyramine, kynuramine and benzylamine did not significantly differ at the various stages of purification (see Table 2), it appears unlikely that the preparative procedure destroyed a more labile component of the enzyme. However, the biphasic nature of the heat inactivation curve of purified platelet MAO compared with that of the more heat stable whole lysed platelet preparation (Fig. 2) indicates that solubilization of the membrane bound enzyme may alter its response to certain physical factors.

The MAO activity of human blood platelets appears to be localized in the mitochondria.[31] However, two other amine oxidases are present in blood; diamine oxidase is found in the plasma in high concentration during normal pregnancy[32] and exists in at least two forms.[33] Human plasma MAO, or benzylamine oxidase[34] possesses properties which differ from those of the other two enzymes.[35] The substrate specificities exhibited by platelet MAO (Tables 2 and 3) are remarkably similar to those of plasma benzylamine oxidase.[35] However, the lack of sensitivity of platelet MAO to semicarbazide and isoniazid distinguishes it from benzylamine oxidase[35] and helps to establish the enzyme as a classical mitochondrial MAO.

Human blood platelet MAO is electrophoretically homogeneous; enzyme from all other human tissues examined to date however exists in multiple forms. Thus, human brain MAO is represented by four isoenzymes,[20] human liver MAO by five[17] and human placental MAO by at least two forms.[16,18] All these isoenzymes differ from each other in their substrate specificities and sensitivities to MAO inhibitors. Thus despite the conclusions of Robinson *et al.*[14] that their properties may be similar, the substrate specificity of platelet MAO appears to be quite unlike that of the enzyme forms found in human or for that matter rat liver.[17] In human whole brain, although benzylamine is most actively deaminated, dopamine is almost as good a substrate;[20] this contrasts strongly with the platelet enzyme where dopamine is the least readily oxidized of the substrates tested. Rat brain MAO is different again and deaminates tyramine more readily than other substrates investigated.[19]

It is not known whether tissue amine levels or other compounds have any effect on MAO activity although evidence is accumulating that this might be so; pretreating rats with DOPA or progesterone increases the activity of two of the three isoenzymes of uterine MAO[36,37] and in rabbits, DOPA administration provokes increased MAO activity in the heart and certain blood vessels.[38] It may therefore be relevant that one of the enzyme forms present in the dopamine-rich[39] human basal ganglia, oxidizes dopamine more than 70 times more readily than an isoenzyme with the same electrophoretic characteristics found in the dopamine-poor cerebral cortex.[20] The closely similar activity pattern of platelet MAO and of plasma benzylamine oxidase might likewise represent an adaptive response to their particular environment. Whilst it is difficult to interpret the substrate preferences of platelet MAO otherwise, paradoxically platelets contain high concentrations of 5-hydroxytryptamine localized within osmiophilic granules:[40] this amine is a poor substrate not only for platelet MAO[14] but also for plasma benzylamine oxidase.[35] Perhaps therefore, the primary role of platelet MAO is not to regulate platelet 5-hydroxytryptamine content which is presumably kept apart from the enzyme by an avid uptake mechanism[41] but to degrade toxic dietary amines which have penetrated the enzymic barriers of the gut and the liver normally responsible for their inactivation.

There is yet another important consideration. The multiplicity of forms of MAO in brain and liver compared with the electrophoretically homogeneous platelet enzyme suggests that the enzymes differ in their molecular configurations. Both beef liver[22] and rat uterine[36, 37] MAO exist as polymeric series whereas preliminary evidence suggests that the differences between the isoenzymes of rat liver MAO may be conformational in character.[42] The molecular weight of human platelet MAO is about 235,000 whereas those of the multiple forms of rat liver MAO lie within the range of 280,000 to 320,000.[42] That of the rabbit liver enzyme is slightly smaller, about 260,000.[43] In contrast, the minimum molecular weight of beef plasma MAO has been estimated as 166,700.[44] The striking differences between MAO isoenzymes from various tissues, not only in form but also in physicochemical properties, makes interpretation of their function difficult. In this context, it is obvious that extreme caution must be exercised in extrapolating the status of human platelet MAO, in health or disease, to other tissues; it also follows that there can be no single *in vivo* test for MAO function as a whole which provides more than an approximate impression of the peculiar properties and functions of specific isoenzymes.

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