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Some properties of particle-bound monoamine oxidase from human whole blood

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Numerous reports have appeared concerning possible correlations between changes in the activity of blood platelet MAO (EC 1.4.3.4) and various pathological states such as schizophrenia [1–3] and depression [4, 5].

However, the characterization of the enzyme is still incomplete and it was therefore of interest to determine its apparent pH optima, K_m and V_{max} as well as responses to clorgyline (a selective inhibitor of MAO-A) and 1-deprenyl (a selective inhibitor of MAO-B) using benzylamine (BA), tyramine (TYR), phenethylamine (PEA), tryptamine (TRYP) and dopamine (DA) as substrates.

The work on the characterization of human blood platelet MAO which has been done so far and reviewed by Murphy and Donelly in 1974 [6] seems to establish that the enzyme is homogenous and has the inhibitor characteristics of MAO-B.

Methods. We have used total particle-bound (presumably mitochondrial) MAO from frozen haemolized human blood in the present investigation, to avoid the more laborious isolation of platelets with accompanying loss of activity. This enzyme preparation apparently consists of a single enzyme, with characteristics similar to, if not identical with, those of the platelet enzyme.

Three portions of human blood, stabilized with citrate and dextrose, were obtained from a blood bank, where they had been stored for up to 1 month at 5° and subsequently stored frozen at −20° until the day of enzyme preparation. The donors differed with respect to age, sex and blood type. Freezing did not reduce MAO activity measurably.

After thawing, the blood was centrifuged four times at 24,000 *g* for 10 min (4°). After each centrifugation the supernatant was discarded and the pellet homogenized in 25 ml sodium–phosphate buffer (0.25 mM, pH 7.0 containing 0.1 mM EDTA). The final mitochondrial suspensions were stored for up to 3 months at −20° until use.

MAO activity was measured by a modification of the method described by Robinson *et al.* [7] and Goridis and Neff [8]. In general: 200 μl of particle-bound MAO suspension was preincubated 15 min at 37°. Then 200 μl of substrate-buffer solution (37°) was added, and the reaction mixture was incubated at 37° for 10–60 min., depending on substrate (10 min for TYR and DA, 30 min for BA and 60 min for TRYP and BA) to give maximum enzyme activity while at the same time maintaining activity as a linear function of time. The reaction mixture contained ^{14}C-labelled substrate (about 200,000 dpm per tube), unlabelled substrate (usually 10^{-4} M), EDTA (50 μM), and sodium–phosphate buffer (12,5 mM). With DA as substrate ascorbic acid was also added as an antioxydant (1 mM). The reaction was stopped by adding 500 μl 1 M perchloric acid.

After cooling on ice for 10 min the tubes were centrifuged 10 min at 2,500 *g*. 500 μl portions of the supernatant were placed on Dowex-50 columns (5 × 35 mm) to bind the remaining amine, and the deaminated products eluted with 2 ml 75% ethanol directly into scintillation vials, which contained 10 ml of an Instagel®–ethanol mixture (9:1). Radioactivity was then measured in a scintillation counter. Less than one percent of the substrate added was converted to product during the incubation time.

Blanks were carried out by pre-incubating the enzyme with deprenyl + clorgyline (0.1 mM, each) or adding perchloric acid before substrate. Assays were performed in duplo.

Results and discussion. Apparent K_m and V_{max} values were determined for all five substrates, at their pH optima, using two portions of blood (Table 1). K_m and V_{max} values were calculated from double reciprocal plots by means of linear regression analysis (mean regression coefficient: 0.997). With PEA as substrate enzyme activity was markedly inhibited at substrate concentrations above 70 μM, and therefore only measurements below this concentration were included in the kinetic analysis. Our K_m and V_{max} values agree well with those of Robinson *et al.* [7].

The response to clorgyline and deprenyl was determined for all five substrates, at their pH optima, using three portions of blood. The enzyme is inhibited by low concentrations of 1-deprenyl (a selective inhibitor of MAO-B), with an average IC_{50} of 6×10^{-8} M and more than 90 per cent inhibition at 4×10^{-7} M with all substrates used. The enzyme is far less sensitive to clorgyline: at a concentration of about 4×10^{-6} M, it is only inhibited 40–70 per cent. The combination of clorgyline and 1-deprenyl (within the same concentration range) had the same inhibitory effect as 1-deprenyl alone. Our results are in agreement with those of Murphy and Donelly [6] and of Edwards and Chang [9].

pH Optima were determined for BA, TYR, TRYP and PEA using three portions of blood. The pHs were adjusted using the following buffers: acetate (pH 4.0–6.0), citrate-phosphate (pH 4.5–7.0), pyrophosphate (pH 5.5–8.5), phos-

Table 1. pH Optima, K_m and V_{max} values for particle-bound MAO from human blood

Substrate	pK_a*	pH optimum	Particle-bound MAO† K_m M × 10^5	Particle-bound MAO† V_{max} (relative)	Blood platelet MAO‡ K_m M × 10^5	Blood platelet MAO‡ V_{max} (relative)
Benzylamine	9.4	8.8	24	100	15	100
Tyramine	9.4	8.2	6	55	5	45
Dopamine	8.9	—	3	37	—	—
Phenethylamine	9.8	6.0	4	19§	—	—
Tryptamine	10.2	7.0	3	7	1.8	11

* Taken from [10].

† The mean of two (serotonin only one) separate determinations, which differed less than 40 per cent.

‡ Results of Robinson *et al.* [7]. Determined at pH 7.5.

§ Extrapolated from linear part of double reciprocal plot.

phate (pH 6.5–8.5), borate (pH 8.0–9.5), carbonate (pH 10.0–10.5) and phosphate (pH 12), all sodium salts. In one series of experiments overlapping buffers were used to detect any possible specific effects of the buffer species. Twenty different buffers with 10–12 different pHs were used in such an experiment for each substrate. No consistent effects of the buffers on MAO activity was observed.

For a given substrate the pH curves were very similar for all three enzyme preparations. The pH optima found for BA, TYR, TRYP, and PEA were near 8.8, 8.2, 7.0 and 6.0, respectively (Table 1). The difference between the pH optima for PEA and BA (6.0 and 8.8, respectively) was quite dramatic and clear-cut: at pH 6.0 MAO activity using BA as substrate was about 5 per cent of maximum activity at pH 8.8. Conversely, at pH 8.8 MAO activity using PEA as substrate was only about 15 per cent of the maximum activity at pH 6.0. Apart from two reports giving the pH optima for serotonin [11] and kynuramine [16], respectively, we have not found any studies concerning the effect of pH on the activity of human blood platelet MAO. However, apparent pH optima for MAO from other species and tissues have been reported by several investigators. Of interest in this context are Alles' [12] results on rabbit liver, which contains almost exclusively MAO-B [13]. He determined pH optima for a series of homologous substrates, and found the same unexpected difference between the pH optima for BA and PEA as we have: pH 8.5 for BA and pH 6.0 for PEA.

Substrate ionization probably cannot explain the difference in pH optima for BA and PEA, since the pK_a values for these two amines differ by less than 0.5 pH units. However, ionization of the enzyme might change the conformation of its active site (or sites), such that substrate preference would be quite different at lower and higher pHs, respectively.

There has been some controversy as to whether the protonated or the unprotonated species of the amines are substrates for MAO in general. McEwen and coworkers concluded from their extensive kinetic studies on MAO from human liver [14, 15], that the unprotonated amine is the substrate for this enzyme. This seems unlikely, in the case of particle-bound MAO from human blood acting on PEA, since the apparent pH optimum for this substrate is more than three pH units below its pK_a value.

Taken together, the facts that the double reciprocal plots, used to calculate K_m and V_{max}, are highly linear and inhibition by the selective MAO inhibitor 1-deprenyl occurs in a single step, make it probable that particle-bound MAO from human blood is a single, homogeneous enzyme and identical to human blood platelet MAO. It cannot be excluded, however, that the particle-bound MAO preparation contains a mixture of iso-enzymes (subtypes of MAO-B) with similar properties.

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REFERENCES

1. R. J. Wyatt, D. L. Murphy, R. Belmaker, S. Cohen, C. H. Donelly and W. Pollin, *Science N.Y.* **179,** 916 (1973).
2. E. Friedman, B. Shopsin, G. Sathananthan and S. Gershon, *Am. J. Psychiat.* **131,** 1392 (1974).
3. H. Y. Meltzer and S. M. Stahl, *Res. Commun. Chem. Path. Pharmac.* **7,** 419 (1974).
4. A. Nies, D. S. Robinson, L. S. Harris and K. R. Lamborn, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes* (Ed. E. Usdin) p. 59. Raven Press, New York (1974).
5. D. L. Murphy and R. Weiss, *Am. J. Psychiat.* **128,** 1351 (1972).
6. D. L. Murphy and C. H. Donelly, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes* (Ed. E. Usdin) p. 71. Raven Press, New York (1974).
7. D. S. Robinson, W. Lovenberg, H. Keiser and A. Sjoerdsma, *Biochem. Pharmac.* **17,** 109 (1968).
8. C. Goridis and N. H. Neff, *J. Neurochem.* **18,** 1673 (1971).
9. D. J. Edwards and S.-S. Chang, *Life Sci.* **17,** 1127 (1975).
10. D. D. Perrin, in *Dissociation Constants of Organic Bases in Aqueous Solutions* (Ed. Butterworths) London (1965).
11. S. Takahashi and T. Karasawa, *Clin. Chim. Acta* **62,** 393 (1975).
12. G. A. Alles and E. V. Heegaard, *J. biol. Chem.* **147,** 487 (1943).
13. R. F. Squires, *Adv. Biochem. Psychopharmac.* **5,** 355 (1972).
14. C. M. McEwen, G. Sasaki and W. R. Lenz, *J. biol. Chem.* **243,** 5217 (1968).
15. C. M. McEwen, G. Sasaki and D. C. Jones, *Biochemistry* **8,** 3952 (1969).
16. M. B. H. Youdim, D. G. Grahame-Smith and H. F. Woods, *Clin. Sci. Molec. Med.* **50,** 479 (1976).