ISOENZYMES OF HUMAN AND RAT LIVER MONOAMINE OXIDASE

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1. Introduction

The existence of multiple forms of monoamine oxidase (E.C. 1.4.3.4) (MAO) has been strongly suspected in the last few years and an impressive body of indirect evidence supporting this view continues to accumulate [1,2]. Apart from two partially successful approaches [3,4], direct physical separation of solubilized MAO into more than one species has only recently been achieved by Youdim and Sandler [5] using electrophoretic methods; their work has since been confirmed [6]. We now wish to report some properties of the different bands of MAO activity isolated from human and rat liver mitochondria in this manner.

2. Experimental

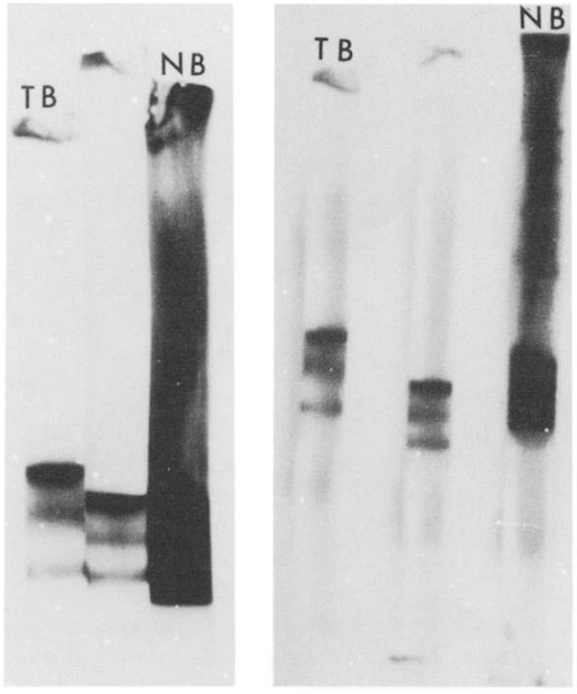
Soluble MAO from human and rat liver mitochondria was prepared by the method of Youdim and Sourkes [7], as modified by Youdim and Sandler [5]. Disc electrophoresis was carried out using the method of Youdim et al. [8]. Samples (0.1 ml) of enzyme solution mixed with Sephadex G-200 (approx. 40 ml/1 g) were applied to the top of 5% (w/v) polyacrylamide gel (Cyanogum 41, British Drug Houses, Ltd.) disc electrophoresis columns (0.5 × 7.5 cm). A continuous 0.05M tris-hydrochloric acid buffer system, pH 8.6, was employed. Samples were applied at the cathode to eight tubes at a time which were run simultaneously for 2-3 hr at room temperature using a constant current of 6 mA per tube. A number of yellowish bands of protein (Naphthalene Black 12B-staining) migrated towards the anode and could be used as markers to localize the MAO bands to which they bore a fixed relationship or coincided. The bands of enzyme activity, whose location had previously been established in earlier experiments by the histochemical procedure of Glenner et al. [9] using tryptamine as substrate, were separated from the gel columns with a sharp razor. Each band was homogenised in about 1 ml 0.05M phosphate buffer, pH 7.4, and after centrifugation at 500 × *g* for 10 min, the supernatant was retained for protein estimation [10] and enzyme assay with the following substrates: ^{14}C-tryptamine and ^{14}C-tyramine [11]; kynuramine [12]; benzylamine [13].

Heat inactivation studies [14] were carried out on the buffered (pH 7.4) enzyme bands at 45°C and activity was measured after 10, 20 and 40 min heating using kynuramine as substrate.

3. Results

Both human and rat liver solubilized mitochondrial MAO consistently separated as four bands of activity (figs. 1A and B) staining blue by the Glenner procedure. These bands were associated with protein as shown by their ability to take up Naphthalene Black 12B. They have been designated H (human) or R (rat) MAO_{1-4} in order of increasing electrophoretic mobility. Thus $HMAO_1$ and $RMAO_1$ remained at the origin whereas $HMAO_4$ and $RMAO_4$ migrated the greatest distance to the anode.

All four human enzyme bands were more heat stable than those of the rat although $RMAO_4$ was



(A) (B)

Fig. 1. Showing polyacrylamide gel disc electrophoresis of solubilized human (A) and rat (B) liver mitochondrial monoamine oxidase. Tetrazolium nitro blue (TB) staining shows 3 bands of monoamine oxidase activity migrating towards the anode and a fourth remaining stationary at the origin. A Naphthalene Black 12B (NB) stain on each preparation gives an indication of total protein applied to the disc.

considerably more resistant to a temperature of 45°C than the other rat isoenzymes (fig. 2).

Relative activities to different substrates are shown in table 1. $HMAO_2$ possessed greater activity against all substrates investigated than other human isoenzymes; kynuramine was more and benzylamine less actively deaminated than other substrates. Although $HMAO_3$ and $HMAO_4$ showed similar relative activities against tryptamine and tyramine, the latter was a better substrate for $HMAO_1$ and $HMAO_2$.

However, the rat enzyme manifested a different pattern. $RMAO_3$ was relatively more active against tryptamine compared with both tyramine and kynuramine, whereas $RMAO_4$ showed relatively greater activity against kynuramine. $RMAO_1$ was almost twice

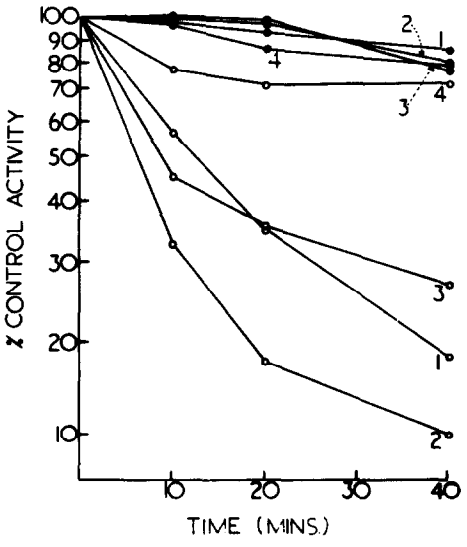


Fig. 2. The effect of heating (45°C) on human (●—●, $HMAO_{1-4}$) and rat (○—○, $RMAO_{1-4}$) liver mitochondrial monoamine oxidase isoenzymes, using kynuramine as substrate.

as active as $RMAO_2$ against kynuramine although both possessed similar activity against tryptamine.

4. Discussion

The data presented here and earlier [5, 8] provide direct confirmation of the view previously arrived at largely by inferential evidence, that there is more than one species of MAO. Kim and D'Iorio [6], who adopted a similar experimental approach, were not able to rule out completely the possibility that the various bands are due to incomplete solubilization of the enzyme and that the observed phenomena stem from one enzyme bound to parts of cellular membranes of various sizes; but as they point out, if fragmentation of the membranes is being observed it is remarkably consistent, and gives rise to similar findings whether sonication or detergent is used for breaking up the mitochondria.

In our hands, the bands are quite constant from preparation to preparation. Any explanation based on incomplete solubilization appears unlikely, for after sonification, the enzyme has always been partially purified and the 30-55% ammonium sulphate preci-

Table 1

The oxidative deamination of various substrates by the 4 electrophoretically separated bands of activity of solubilized human (H) and rat (R) liver mitochondrial monoamine oxidase (MAO_{1-4}). Each figure represents nanomoles deaminated product formed per 10 min per mg protein and is the mean ± S.E.M. of 3-7 experiments.

		$HMAO_1$	$HMAO_2$	$HMAO_3$	$HMAO_4$
Human	Tryptamine	8.03 ± 1.20	14.40 ± 1.60	2.44 ± 0.71	0.51 ± 0.08
	Tyramine	26.50 ± 4.40	38.70 ± 4.50	3.75 ± 0.85	0.55 ± 0.04
	Kynuramine	54.60 ± 4.78	114.20 ± 10.44	13.10 ± 2.15	4.51 ± 0.88
	Benzylamine	1.55 ± 0.47	2.07 ± 0.61	0.027 ± 0.01	0.37 ± 0.09
		$RMAO_1$	$RMAO_2$	$RMAO_3$	$RMAO_4$
Rat	Tryptamine	5.40 ± 0.65	5.18 ± 0.40	5.48 ± 0.43	0.73 ± 0.27
	Tyramine	10.20 ± 0.41	6.37 ± 0.24	1.10 ± 0.11	0.56 ± 0.07
	Kynuramine	21.20 ± 3.42	11.60 ± 2.84	2.41 ± 1.00	1.33 ± 0.53

pitate is readily soluble in aqueous media without the use of detergents. Centrifugation at 170000 × *g* for 2 hr does not reveal any sediment. The bands show differing substrate specificity and heat stability and in some preliminary experiments [8] (in preparation) show differing sensitivity to MAO inhibitors and pH-activity curves. It therefore seems reasonable to call them isoenzymes.

Whereas Kim and D'Iorio [6] used cellulose polyacetate membrane electrophoresis, we employed the more sensitive polyacrylamide gel. This difference in separating ability probably explains our finding of three rat liver isoenzymes migrating towards the anode compared with the two they observed. On the other hand our MAO which remained at the origin is probably composite, for Kim and D'Iorio were able to detect two bands moving towards the cathode during their experiments.

Although the human liver mitochondrial isoenzymes appear similar to those of the rat, there are quite distinct substrate specificities and heat inactivation patterns. All the human bands are relatively stable at 45°C, but only $RMAO_4$ has a similar stability. It is of interest that two other groups [14,15] have also noted a heat stable MAO fraction in rat liver.

With the possible exception of tyramine, all the substrates used in the present work are unlikely to be of physiological importance. Work is now in progress using the endogenous amines, noradrenaline and 5-hydroxytryptamine as substrates for isoenzymes from mitochondria and microsomes from different organs and from different species.

Acknowledgements

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