PURIFICATION AND SOLUBILIZATION OF MONOAMINE OXIDASE OF RAT LIVER MITOCHONDRIA

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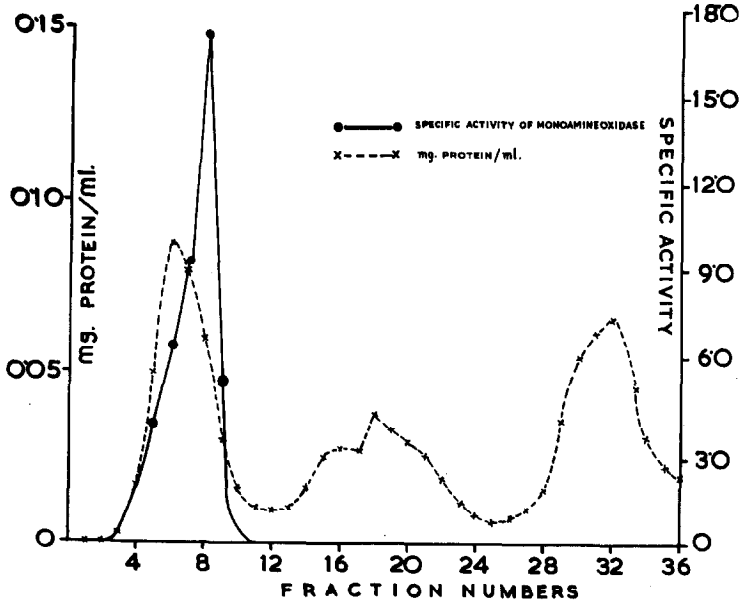
Several attempts have been reported to solubilize mitochondrial monoamine oxidase (MAO) but the different approaches made to purify the enzyme from mitochondrial extracts have been unsuccessful (Blaschko and Jacobson, 1942; Barsky et al., 1953; Cotzias et al., 1954; Zeller et al., 1955; Kobayashi et al., 1955; Harris, 1960). Barbato et al.(1963) achieved a 20-fold purification of the enzyme from beef liver but the preparation was insoluble and easily sedimentable. Gorkin (1963) reported the preparation of a partially purified MAO from rat liver mitochondria. Preparation of a soluble MAO by sonication of mitochondria has also been recently described (Coq and Baron, 1964). The extracts were, however, not used for any further purification. Difficulties encountered in solubilizing mitochondrial MAO have thus stood in the way of preparing the enzyme in a highly purified form for kinetic and inhibition studies.

A comparatively simple method for preparing a 350-fold purified MAO from rat liver mitochondria is described in this communication. Rat liver mitochondria prepared in isotonic sucrose according to the method of Schneider and Hogeboom (1950) were once washed and

resuspended in 0.01 M phosphate buffer pH 7.6 and exposed to ultrasonic waves at 25 kc/s (output amperage 2.5) for 25 minutes in a Mullard Magnetostrictor Generator. The suspension was placed in a stainless steel flask fitted to the transducer element and the flask and contents during exposure to sound waves were kept chilled. The resulting suspension was centrifuged at 4,050,000 g-minutes (de Duve et al., 1953) in a Servall cold centrifuge at + 2° and the supernatant was found to contain over 90 % of the original activity of mitochondria.

Aliquots of the supernate corresponding to 40 mg protein were applied directly to a DEAE cellulose column (30 x 2.5 cm) equilibrated with 0.01 M phosphate pH 7.6, and containing sodium chloride to a concentration of 0.01 M. The enzyme was eluted by stepwise addition of four lots of 100 ml 0.01 M phosphate buffer pH 7.6 containing 0.01,

FIG.1.
ELUTION PATTERN OBTAINED BY COLUMN CHROMATOGRAPHY ON D E A E CELLULOSE



0.05, 0.1 and 0.5 M sodium chloride. All the above steps were conducted at 8°. The active fractions (10 ml each) were found to be water clear or pale yellow in colour. A typical elution profile is given in Fig. 1 from which it would be clear that all the MAO activity is recoverable in the initial few fractions at low sodium chloride

Table I

Steps in the purification of MAO of rat liver mitochondria

Preparations	Specific Activity μg NH_3/mg protein	Purification (fold)
Homogenate	0.32	1.0
Intact mitochondria	0.68	2.1
Supernate of sonicated mitochondria	3.54	11.0
Residue of sonicated mitochondria	0.38	1.1
First DEAE cellulose eluate	17.16	53.6
Second DEAE cellulose eluate	114.26	357.0

Enzyme activity was assayed in 0.05 M phosphate buffer pH 7.6 in the presence of 30 μ moles of tyramine hydrochloride, neutralised before use. Incubations were carried out for 30 mins at 37°C and ammonia estimated directly by nesslerisation or after diffusion by the manometric technique of Braganca et al. (1954). All values are corrected for blanks. Protein concentration was determined spectrophotometrically at 280 mμ (Warburg and Christian, 1942).

concentration. The active fractions were pooled, lyophilised and rechromatographed on DEAE cellulose. The fractions emerging at a NaCl concentration of 0.01 M contained the enzyme which showed on specific activity basis an overall purification of 350 fold (Table I).

Table II

Comparative activities of different enzyme preparations upon various monoamines

Substrate	MAO activity (μg NH_3/30 mins)		
	Supernate of sonicated mitochondria	Eluate after first chromatography	Eluate after second chromatography
Tyramine hydrochloride	5.2 (100)	6.9 (100)	7.2 (100)
Tryptamine hydrochloride	2.6 (50)	3.0 (43)	3.4 (47)
5-hydroxy tryptamine creatinine sulphate	2.0 (38)	2.4 (34)	2.2 (30)
Adrenaline	1.2 (23)	2.0 (28)	1.6 (22)

Eluate fractions with high specific activity obtained after the first and second chromatography were pooled and concentrated by lyophilisation before use. Protein concentrations used in the assay systems were 1.5, 0.5 and 0.105 mg for supernate of sonicated mitochondria , eluate after first chromatography, and eluate after the second chromatography respectively. The substrate concentrations employed in a total volume of 2.0 ml in μ moles: tyramine hydrochloride -30.0; tryptamine hydrochloride -25.0; 5-hydroxy tryptamine creatinine sulphate -25.0; or adrenaline -18.2 . All the substrates were neutralised just before use. Incubations were made for 30 mins at 37°C in Warburg's apparatus and ammonia estimated according to Braganca et al. All values are corrected for blanks. Other details are same as in Table I. Figures in parentheses indicate relative activity with respect to tyramine as 100.

A comparison of the activities of the mitochondrial sonicate, the first and second DEAE eluates tested on four monoamines is set out in Table II. Further purification of this solubilized preparation of rat liver MAO and the application of this method to other tissues like rat brain and tissues of other animals is in hand.

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