ISOLATION AND CHARACTERIZATION OF MONOAMINE OXIDASE FROM HYPERFUNCTIONING HUMAN THYROID

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Summary: Human thyroid monoamine oxidase from hyperfunctioning thyroids was isolated and purified by separating the mitochondria by differential centrifugation followed by ultrasonication. The suspension containing the active enzyme from the DEAE-column, on passing through a Sephadex G-200 column gave three peaks corresponding to molecular weights of approximately 220,000, 23,000 and 4000 of which the component with molecular weight 4000 showed enzyme activity. The blood of the patients was analyzed for plasma MAO, RBC cholinesterase, plasma histaminase and plasma catecholamines. Histology and histochemistry of the thyroid tissues were also done. The data are examined from the point of view of the prevalent idea of the possible existence of MAO in multiple forms.

Key words: monoamine oxidase human thyroid

INTRODUCTION

The enzyme monoamine oxidase (MAO) catalyzes the oxidative deamination of monoamines such as 5-hydroxytryptamine, dopamine and noradrenaline, which are considered to be neurotransmitters in the central nervous system. The biochemical nature of the enzyme and its physiological role have been reported (7, 33). That there is an inter-relationship between stresses of various kinds and the nature and level of MAO in the thyroid gland is also well known. All these aspects have been recently reviewed with special reference to MAO in health and disease (34). Extensive studies on MAO from animal tissues such as beef liver (13) and rat tissues (9,14,19,31, 38) have been reported. In contrast to the studies reported above, reports on MAO from human tissues have been rather scanty. Youdim and Sandler (37) reported studies on human placental MAO. Collins *et al.* (5) have shown the multiple forms of human brian MAO. Studies on human plasma MAO have been reported by McEwen (24,26). More recently, Norstrand and Glantz (18) have purified human liver MAO and reported some of its properties. About the only recently available study on human thyroid is that by Hidaka *et al.* (17), where estimates of MAO activity levels have been made in hyperfunctioning, hypofunctioning and malignant human thyroids.

The present paper outlines the isolation, purification and partial characterization of human thyroid MAO from hyperfunctioning thyroids. The question regarding the possibility of the enzyme existing as isoenzymes or in multiple forms has also been examined. Estimates of plasma histaminase and catecholamines in blood of patients are also reported. Studies described below

will be expected to throw light on the role of MAO in thyroid disorders, because it is believed that there exists an inter-relationship between stresses of various kinds and thyroid disorders.

Portions of the material contained in this paper have already been reported in an abbreviated form (35).

MATERIALS AND METHODS

Tissues: Human thyroid tissues were collected just after the operation and were immediately immersed in ice-cold 0.25 M sucrose solution. The tissues were stored at -18°C.

Chemicals and reagents : DEAE-cellulose (anion-exchanger of medium mesh), Sephadex G-200 (40-120 micron), tyramine hydrochloride, semicarbazide hydrochloride, riboflavin, tryptamine hydrochloride, kynuramine dihydrobromide, bovine serum albumin, cytochrome C, gamma globulin, trypsin, bovine serum albumin and glycine were from Sigma Chemicals, U.S.A. Tris (hyroxymethyl aminomethane) and TCA were from E. Merk, Germany. TEMED, acrylamide, ammonium persulphate and bromophenol blue were from B.D.H, Poole, England. All other chemicals for preparing buffers and reagents were of analytical grade and were from B. D. H. Chemicals, India. The water used for preparing buffers and reagents was first deionized and then triple-distilled.

Preparation of crude monoamine oxidase : A flow sheet detailing various steps in the preparation of monoamine oxidase from the tissues is shown in Fig. 1. It is necessary at this point to emphasize that the yield was not aimed to be measured. The present studies are more aimed at getting information on the nature of the enzyme from hyperfunctioning human thyroids, rather than on aspects such as yield.

DEAE-Cellulose chromatography : This was carried out according to the method of Guha and Krishnamurthy (16) with minor modifications. The preparation of DEAE-cellulose for use in the column was according to Peterson and Sober (29). The glass column was jacketed which facilitated passing ice-cold water to keep the column and its contents at a temperature of 5°C. The column, after being packed with the gel, was first washed and equilibrated with 0.01M phosphate buffer, pH 7.6 and then with 0.01M phosphate buffer, pH 7.6 containing NaCl to a concentration of 0.01M . The enzyme solution was eluted with the latter buffer and fractions of 3 *ml* were collected in tubes kept immersed in ice. The O.D's at 280 nm were measured spectrophotometrically and fractions were tested for enzyme activity using tyramine HCl as the substrate. The active fractions were pooled together and concentrated by dry dialysis against sucrose. The concentrated enzyme solution was then rechromotographed on another DEAE column in a manner exactly the same as that outlined above. 3 *ml* fractions were collected, tested for enzyme activity and the active fractions were pooled. The reason for this rechromatography has been explained by Guha and Krishnamurthy (16). They have explained that in the case of MAO, this procedure results in a higher purity of the enzyme.

Sephadex gel filtration : The active enzyme solution obtained above was concentrated by dialysis against dry sucrose and eluted from a Sephadex-G-200 column with 0.01M phosphate buffer, pH 7.6. The elution profile obtained from this study is shown in Fig. 2. The

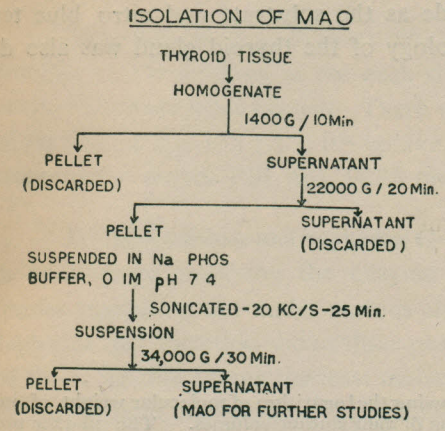

Fig. 1: Flow chart showing the steps in the extraction of MAO.

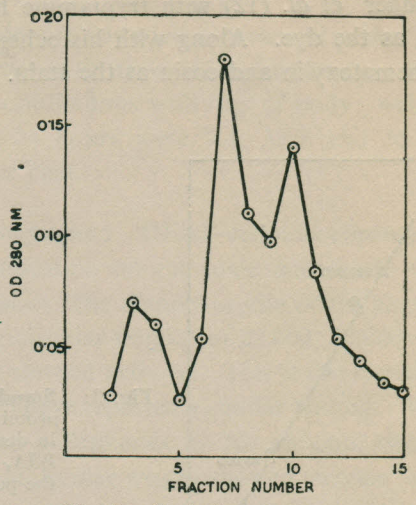

Fig. 2: Sephadex G-200 elution profile of MAO sample obtained after elution through the DEAE-cellulose column.

peak fractions were tested for enzyme activity. The Sephadex column was standardized by eluting a mixure of marker proteins. The standard graph with molecular weight as function of elution volume is shown in Fig. 3. This graph was used for estimation of molecular weight of fractions.

Polyacrylamide gel electrophoresis : Gel electrophoresis was done according to the method of Davis (6), using the enzyme stain of Robinson (20). Electrophoresis was carried out at a temperature of 4°C with a current flow of about 4mA per tube for about two hours.

Enzyme assay: Enzyme assay was done by one of two following methods. In the first, tyramine hydrochloride was used is the substrate according to the method of Green and Haughton (15). In the second method which is more rapid, kynuramine dihydrobromide was the substrate employed. This method according to Weissbach *et al.* (36), measures the change in absorbancy at 360 nm per unit time of the enzyme-substrate mixture.

Protein estimation : The protein content was estimated according to the method of Lowry *et al.* (23).

Absorption spectrum : Absorption spectra were obtained of the active fractions from the Sephadex column with the help of a Cary 14 recording spectrophotometer.

Histology and Histochemistry : Histochemistry of MAO was done according to the method of Glenner *et al.* (12) with tryptamine hydrochloride as the substrate and nitro blue tetrazolium as the dye. Along with histochemistry, histology of the thyroid gland was also done using hematoxylin and eosin as the stain.

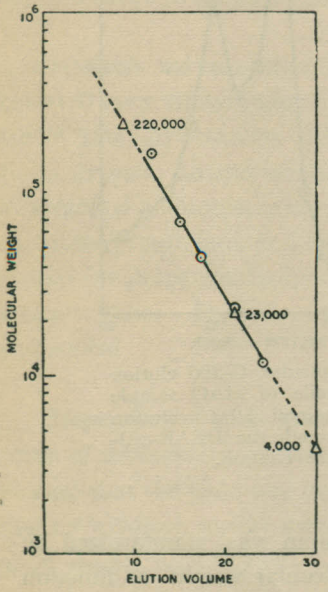

Fig. 3: Standard graph showing the logarithm of molecular weight of marker proteins as a function of their elution volumes. The marker proteins in descending order of molecular weight are : Gamma globulin, BSA, ovine serum albumin, trypsin and cytochrome C. △represents the position of the MAO fractions.

Blood analysis : Blood was obtained from the patient just before the operation. In all, 12 *ml* of blood was collected. Of this, 8 *ml* was heparinized for plasma and 4 *ml* was used for getting serum. In the plasma, estimation of catecholamines, histaminase, MAO and RBC cholinesterase were done. The serum was examined for serum PBI. The plasma catecholamines were estimated colorimetrically (11). Spectrophotometric method was employed for the estimation of plasma histaminase (1), plasma MAO (25) and RBC cholinesterase (21). Serum PBI was estimated by the alkaline incineration method (2).

RESULTS

Case reports of the patients taken up for study are given below :

Case 1 : R. D., 18 years old, married, female was admitted as a case of primary thyrotoxicosis; she had swelling in the neck since three years, with increased apetite and intolerance

to heat; she had taken antithyroid drugs since one year. The gland moved with deglutition and she had difficulty in swallowing since six months. Size of the gland was 2″x 2″. Eye signs were normal and tremors were present. Her pulse rate was 98 per minute; I^{131} uptake for 1,4 and 24 hours were 27.55%, 59.71% and 68.2% respectively. The serum PBI was 12.4 microgram per cent. There was no change in the weight of the body.

Case 2 : N. N., 36 years old, married, male was admitted as a case of primary thyrotoxicosis; he had swelling in the neck since four years with nervousness and anxiety since three years. Tremors were present. There was mild exophthalmus with loss of body weight. Size of the thyroid was 1″ x 1.5″ ; I^{131} uptake for 1,4 and 24 hours were 9.4, 33.9 and 55.9 percent respectively; serum PBI was 14.25 microgram per cent.

DEAE-Cellulose and Sephadex G-200 chromatography : DEAE-cellulose chromatography, in addition to purifying the enzyme provides indication on the ionic nature of the active species separating out under various elution volumes. The elution profile of the enzyme from Sephadex gel filtration shows three peaks having molecular weights of 22,000; 23,000 and 4,000. Of these, the one having the least molecular weight showed activity. This is not exactly surprising and without precedence. Mushahwar *et al.* (27) have reported a similar finding. When they passed an aliquot of DEAE-cellulose effluent through a Sephadex G-200 column, two proteins peaks were found of which the one having a lesser molecular weight showed enzyme activity.

Polyacrylamide gel electrophoresis : Analysis of the crude enzyme by this method revealed one active band in agreement with the gel filtration data reported above.

Ultravoilet absorption spectrum : The spectrum was similar to that of a normal protein and is more or less the same as that reported from human liver by Norstrand and Glantz (28).

Histology and Histochemistry : These analysis confirmed the hyperfunctioning character of the thyroid glands taken up for these studies.

Changes in blood neurohumours : Table I shows that the data obtained in the present studies in this regard are not very definitive. Obviously a large number of patients will have to be examined to get meaningful data in this respect.

TABLE I

Sl. No.	*Name*	*Diagnosis*	*Change in blood neurohumours*				*Thyroid function serum PBI (in microgram %)*
			RBC Cholinesterase (in P.U.)	*Plasma catecholamines (in microgram/ml)*	*Plasma histaminase (in P.U./ml)*	*Plasma MAO (in P.U./ml)*	
1.	R.D.	Thyrotoxicosis	120	10.8	90	17	12.4
2.	N.N.	Thyrotoxicosis	105	29.9	285	7	14.3
3.	Normal	Control	80±20	12-20	105±32		4-8

DISCUSSION

This paper is an attempt at a partial characterization and subsequent understanding of the nature of the enzyme MAO from hyperfunctioning human thyroids. An interesting observation from the Sephadex gel filtration is that only one of the subunits is found to be active. The present data can be examined from the point of veiw of the possibility of multiple forms of MAO. Alles and Heegard (3) as early as 1943 studied liver MAO activity in different species and concluded that there was a strong evidence for the existence of the enzyme in different forms. Borges and D' Iorio (8) examined rat liver mitochondrial MAO's and found that serotonin and tyramine had a different behaviour with MAO as compared to benzylamine. Squires (32) indicated that there were atleast three forms of mitochondrial MAO in mouse. Youdim and Sandler (37) and Collins *et al.* (4), on the basis of their experiments claimed to have a confirmation of the existence of multiple MAO's and they designated them as isoenzymes. More recently Lin and Castell (22) isolated different forms of human MAO which they called isoenzymes. According to them, the three forms have the same molecular weight of 150,000 the difference among the three being their different substrate specificities and thermal stability. Kroon and Veldstra(20) went to step further to suggest that the multiple forms have a definite physiological meaning to accommodate the possibility of deaminating different physiologic amines. This statement acquires added significance in view of the finding that the multiple forms of MAO have characteristic anatomical distribution, each with its own substrate specificity and sensitivity to inhibitors (10,18). The findings in the present studies are in general agreement with the observations made above. We do find three forms of different molecular weight of which only one is specific to the substrate used.

In general therefore, it can be said that the data available till to date support the view that MAO could exist in multiple forms of the same enzyme. Our present findings tend to support this view point.

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