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Isoenzyme Composition of Human Plasma Monoamine Oxidase in Normal Subjects and in Fibrotic Liver Disease (39138)[1]

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The clinical diagnosis of cirrhosis of the liver is often difficult since there is no direct test of liver function that allows early detection of the development of hepatic fibrosis. Studies from different laboratories have shown that plasma monoamine oxidase (MAO) activity is often increased in patients with chronic firbrotic liver disease (1–3). Thus, the elevation of MAO activity, if characterized and understood, may have clinical implication for early diagnosis of cirrhosis of the liver.

It has been reported previously that MAO isolated from pooled normal human plasma and from patients with hemochromatosis and hepatic fibrosis have certain intrinsic molecular differences (4), namely, different pH optimum ranges and different kinetic properties in the presence of inhibitors. Recently, three distinct activity peaks of MAO from normal plasma have been separated by hydroxyapatite (HA) column chromatography (5). These three chromatographically different forms of MAO were found to have different thermal stability, substrate specificity, and inhibition response by semicarbazide. We suggest that the three forms may represent isoenzymes of MAO.

In this communication, we report that MAO isolated from two patients with hemochromatosis and hepatic fibrosis (abn-MAO) and MAO from pooled normal human plasma (n-MAO) have different compositions as revealed by HA column chromatography. Also a form of MAO absent in normal plasma was found in abn-MAO.

Materials and methods. Benzylamine-free base was purchased from Matheson Coleman and Bell Manufacturing Chemist. It was redistilled under vacuum before use. Semicarbazide hydrochloride was purchased from Fisher Scientific Company. Recrystallized ammonium sulfate was obtained through Schwarz/Mann. Hydroxyapatite Suspension (Type I) was purchased from Sigma Chemical Company.

Activity of monoamine oxidase. MAO activity was assayed by direct measurement of benzaldehyde formation from benzylamine (6). One unit of enzyme was defined as the amount of the enzyme catalyzing a change in absorbance of 0.01 per 90 min at 250 nm at 37°. The reaction mixture contained 20–100 units of enzyme, 2.2 m*M* benzylamine, 0.2 *M* phosphate buffer, pH 7.2 in a total volume of 1.5 ml (5). The molar absorptivity of benzaldehyde at 250 nm was assumed to be $0.12 \times 10^4\ M^{-1}\ cm^{-1}$ (7). One unit of enzyme corresponds to the formation of 1.25 nmoles of benzaldehyde in 90 min at 37°. Protein was either estimated by the optical density at 280 nm or by the method of Lowry *et al.* (8) with bovine serum albumin as standard.

Preparation of enzyme for HA column chromatography. Plasma was obtained from two patients with biopsy confirmed hemochromatosis and hepatic fibrosis during biweekly phlebotomy treatment. Pooled normal human plasma were obtained through a blood bank. Normal plasma was matched with patients according to the storage period at −20°. No appreciable difference in stability was found over a range of 4 months of storage.

The plasma was separated from heparinized whole blood by centrifugation at 800*g* for 10 min (4°). It was immediately frozen and kept in −20° until enough material (4–6 units) were collected for one purification. The total plasma MAO level of both patients was elevated, ranging from 1.5 times (patient A) to 3 times (patient B) higher

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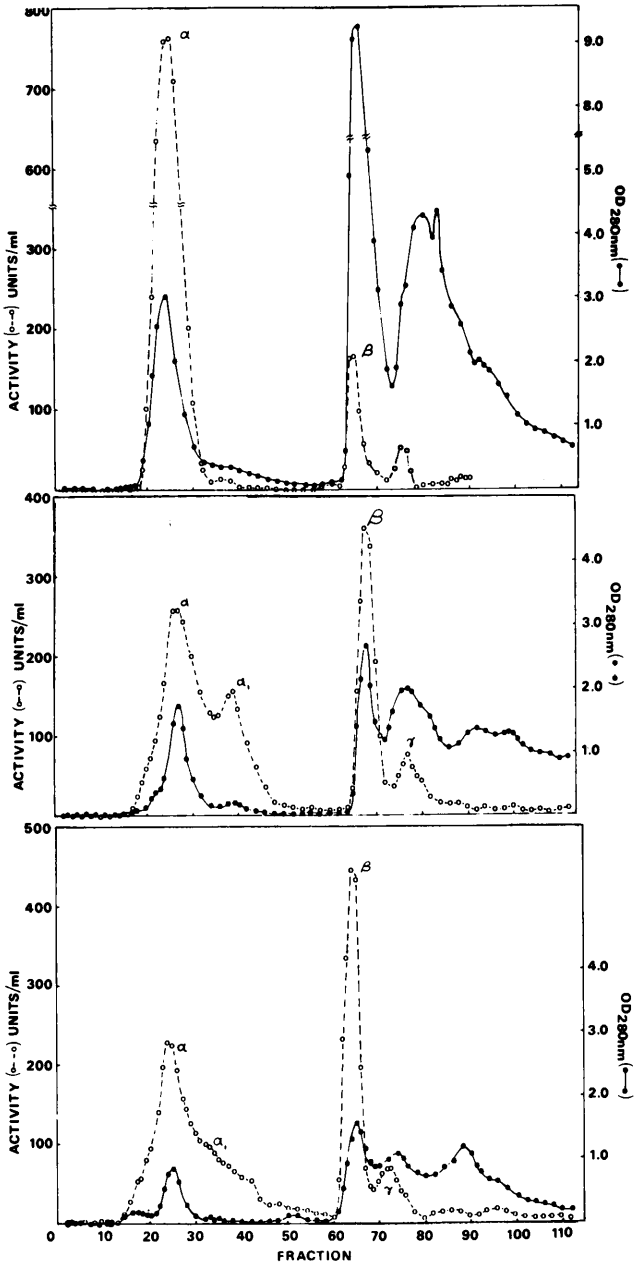

FIG. 1. Hydroxyapatite column chromatograph of human plasma MAO. A typical normal MAO chromatogram and two patients with fibrotic liver disease and hemochromatosis. In every case MAO was first partially purified (15). MAO of specific activity of 60, 67, and 200 for normal, patient A, and patient B, respectively, were applied to a 16 × 116-mm column. Alpha form (α) was eluted with starting buffer; β and γ were eluted with convex gradient generated by adding high concentration phosphate buffer (0.1 *M*) to a constant volume (27 ml) of low concentrate phosphate buffer (0.002 *M*). The conductivity of eluent were monitored with a Westcane conductivity meter. The concentrations of the phosphate buffer at which MAO activity was detected were: α, 2 m*M*; β, 20 m*M*; γ, 40 m*M*. (O—O) activity, and (●—●) $O.D._{280}$.

than the normal mean value [27.1 ± 0.8 McEwen units (10)].

MAO was first purified to 200-fold or higher as described in detail earlier (4). The fractions that precipitate between 27 and 31 g of solid ammonium sulfate/100 ml of enzyme solution were used for HA column chromatography. These fractions represent the fractions of highest specific activity.

Results and discussion. Chromatography on HA column at pH 6.8 resolved n-MAO into three activity peaks designated as α, β, and γ. The α form was eluted by the starting buffer (2 m*M* phosphate buffer), whereas β and γ forms were eluted at higher concentration of the buffer, 20 and 40 m*M*, respectively.

In normal plasma the majority of MAO activity (about 84%) is in α form (Fig. 1). However, under the same conditions, the elution profiles of abn-MAO from the patients were different: the α form was drastically reduced and the β form was sharply elevated. In the chromatogram of patient A there is an additional peak designated as α_1, which was eluted by the starting buffer immediately following the α peak. In patient B's chromatogram, at the same position as α_1, a shoulder was observed. This is distinctly different from n-MAO where the α peak is always sharp with no tailing.

For the purpose of comparison, all three columns were adjusted to the same size (16 × 116 mm) and similar amounts of total MAO activity were applied (15,000 units) on each column. The yields of the columns were all about 56%. Table I summarizes the distribution of the three forms in n-MAO and in abn-MAO. The α form was reduced from 84% in normal to 38% in patient A and 33% in patient B. The β form was elevated from 10% in normal to 24% in A and 30% in B. The γ form did not show a significant change.

It is noteworthy that the flow rate of the HA column was determined only by the size of HA particles and the column length. Increasing the pump pressure had little effect on the flow rate. At pH 6.8, MAO is not very stable and the column eluent loses about 15% activity per day by standing at 4°. Therefore, delay in assay or change in column length will affect the relative yield of each form. However, by keeping all conditions constant, the chromatographic profiles are found to be very reproducible. Over six runs of column chromatography of n-MAO, the mean percentage of the α form is 75.6 ± 9.0% (± SD), the β form is 12.3 ± 2.7%, and the γ form is 6.2 ± 1.5%, while α_1 was never observed. The reproducibility in abn-MAO was also good. With three experiments in patient A and two experiments in patient B the mean percentages of the α form were 35.0 ± 2.6 and 33.7 ± 0.9%, respectively, whereas for the β form they were 22.4 ± 2.3% and 27.3 ± 2.8%, respectively. The α form was 7.2 ± 2.5% for both A and B.

Since the total plasma level of MAO activity is different in patients and normal plasma, comparison was also made on the basis of units/liter of plasma. This was calculated by dividing the activity of each form by the volume of plasma used for that column. Table II is a summary of these calculations. It is obvious from this calculation that the most remarkable differences between normal plasma and the patient's plasma are the elevation of the β form (4- to 10-fold) and the presence of α_1. The γ form was not compared because of low recov-

TABLE I. SUMMARY OF HYDROXYAPATITE COLUMN CHROMATOGRAPHY.

	Units put on column	Total units recovered[a]	Yield (%)	Isoenzyme Fraction[b]								Plasma used (ml)
				α		α_1		β		γ		
				(units)	(%)	(units)	(%)	(units)	(%)	(units)	(%)	
Normal	15,800	8420	53.3	7140	84.8	—	—	810	9.6	460	5.5	1426
Patient A	15,012	8838	59.9	3348	37.9	1624	18.4	2113	23.9	667	7.6	1091
Patient B	14,950	8619	57.7	2827	32.8	1839	21.3	2551	29.6	601	7.0	440

[a] Total units recovered = sum of total activity recovered from all fractions in Fig. 1.
[b] Activity of isoenzyme = sum of total activity of the fractions under each activity peak in Fig. 1.

TABLE II. ISOENZYME COMPOSITION IN 1000 ml OF PLASMA

	Plasma used for HA column (ml)		
	Normal, 1426	Patient A, 1091	Patient B, 440
	MAO (units/liter of plasma)		
α	5007.0	3069.7	6425
α_1	—	1488.5	4179.5
β	568.0	1936.7	5797.7
γ	322.6	611.6	1367.5

ery. However, one must not confuse the isoenzyme level per liter of plasma in Table II with the actual units of the isoenzyme in intact plasma. Table II shows the composition of purified enzyme but not the composition in the plasma.

In previous clinical studies, virtually all patients with liver disease and elevated levels of total plasma MAO have been shown to have hepatic fibrosis. However, many patients with cirrhosis have total MAO levels in a normal range (1, 3). In patient A, although total MAO elevation is marginal, the existance of α_1 and the elevation of β are unmistakable. These findings suggest that enzyme patterns showing these two features may be a better indication of hepatic fibrosis than the total MAO level.

The two paitents in this study both have hemochromatosis with cirrhosis; therefore, we cannot rule out the possibility that the results observed are due to tissue iron deposition only. Further studies on total plasma MAO level and isoenzyme composition in hemosiderosis and also isoenzyme patterns in other forms of cirrhosis are needed.

Summary. Hydroxyapatite column chromatography elution profile reveals characteristic differences between monoamine oxidase (MAO) isolated from normal human plasma and from patients with hemochromatosis having hepatic fibrosis. In normal plasma, the α form constitutes about 84% of the enzyme, with the remainder in the β and γ forms. By contrast, in hemochromatosis there is less α form (less than 40%), an additional α_1 form (about 20%) which was eluted immediately after α form, increased β form (more than 25%), and no significant difference in γ form. When calculated on the basis of total amount per liter, hemochromatosis is characterized by elevation of β form (3- to 10-fold) and the presence of α_1. These results also appear to indicate that the mulitple forms separated by hydroxyapatite column chromatography represent true multiplicity of human plasma MAO *in vivo*.

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