

Heterogeneity of Human Platelets. VII. Platelet Monoamine Oxidase Activity in Normals and Patients With Autoimmune Thrombocytopenic Purpura and Reactive Thrombocytosis: Its Relationship to Platelet Protein Density

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The level of platelet monoamine oxidase (MAO) activity has been used as a genetic marker for certain mental disorders. The purpose of this study was to investigate the influence of several nongenetic factors on platelet MAO activity. Platelet MAO, platelet count, mean platelet volume, percent megathrombocytes, platelet protein, and platelet protein density were determined in 19 normal subjects and in patients with two clinical conditions associated with extremes in platelet count and mean platelet volume: 6 patients with autoimmune thrombocytopenic purpura (ATP), characterized by low platelet count and increased mean platelet volume; and 6 patients with reactive thrombocytosis (RT), characterized by high platelet count and decreased mean platelet volume. Platelet protein density was expressed as total platelet protein/total platelet volume. Patients with ATP, when compared to normal subjects, had 2.3-fold lower platelet protein density and 2.1-fold lower specific activity of MAO. Patients with RT,

when compared to normal subjects, had 1.9-fold higher platelet protein density and 1.5-fold higher specific activity of MAO. Platelets from RT patients, when compared to ATP patients, had 4.3-fold greater protein density and 3.2-fold higher specific activity of MAO. Platelets from normal subjects were separated into five different platelet density fractions. Platelet MAO and protein content were found to be heterogeneously dispersed. The extreme heavy platelet fraction had 15-fold greater specific activity of MAO and 18-fold greater protein content, compared to the extreme light platelet fraction. We conclude that high specific activity of MAO is associated with platelets densely packed with protein. Accordingly, nongenetic factors such as changes in platelet number, volume, and protein content, which could be induced by stress, blood loss, iron deficiency, or other conditions, could contribute to changes in protein density, and consequently affect the specific activity of platelet MAO.

THE LEVEL of platelet monoamine oxidase (MAO) activity has been employed as a genetic marker for several abnormal mental conditions.¹⁻⁷ The purpose of this investigation was to determine the influence of several nongenetic factors on the activity of platelet MAO.

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In this report we present evidence that platelet MAO activity and protein content are heterogeneously dispersed in normal subjects. We have also studied platelets from patients with two pathophysiologic conditions characterized by extremes in platelet count. Platelets from patients with autoimmune thrombocytopenic purpura (ATP), characterized by decreased platelet count, increased platelet turnover, and large platelets,⁸⁻¹² have been studied for platelet MAO and protein density and are compared to platelets from patients with reactive thrombocytosis (RT), characterized by increased platelet count, increased platelet turnover, and small platelets.¹³

MATERIALS AND METHODS

Platelet count, percent megathrombocytes, platelet protein, particulate platelet protein, mean platelet volume, and the specific activity (SA) of MAO, using two substrates, benzylamine and tryptamine, were determined in platelets from each of 19 normal subjects, 6 patients with ATP, and 6 patients with RT. Blood samples from patients with a diagnosis of ATP or RT were obtained from the Hematology Service of New York University Medical Center. Normal subjects were laboratory personnel and medical student volunteers.

Platelet counts were performed manually under phase contrast optics on whole blood specimens anticoagulated with EDTA (0.5% final concentration). Percent megathrombocytes and mean platelet volumes were measured from the same blood specimen using a Coulter Counter model B as described previously.^{8,14,15} Platelets were isolated as follows: Approximately 25 ml of venous blood was drawn and placed into a 50-ml Nalgene centrifuge tube containing 3 ml of 5% EDTA and mixed. Four 5-ml portions were accurately pipetted into polycarbonate tubes and platelet-rich plasma (PRP) was obtained by centrifuging at 100 g for 20 min at 4°C. The PRP was carefully transferred to another centrifuge tube using siliconized pipettes. Platelets were harvested by centrifuging at 2500 g for 30 min. The plasma was removed, and the platelet pellets combined corresponded to 20 ml of whole blood. The combined platelet pellet was resuspended in 5 ml isotonic saline and resedimented at 10,000 g for 10 min. The resulting platelet pellet was then lysed by homogenization for 3 min in a ground-glass microhomogenizer in 1 ml of distilled H₂O.

A portion of the resultant platelet homogenate was assayed for total protein and the remainder was centrifuged at 100,000 g for 10 min in order to obtain the particulate fraction, including mitochondria, which contained 99% of the MAO activity. The platelet particulate pellet was then homogenized in 0.3 ml of 0.05 M phosphate buffer, pH 7.4. The platelet homogenate, containing both soluble and particulate protein, and the final particulate fraction were assayed for protein according to the method of Lowry et al.,¹⁶ to obtain platelet protein and platelet particulate protein, respectively.

The distribution of MAO in five different platelet density fractions was obtained by differential centrifugation.¹⁵ The PRP from nine subjects of the normal group was subjected to sequential centrifugation at 150, 250, 450, and 1000 g, and the pellets were harvested. Each platelet pellet was then prepared for MAO assay and protein determination as described for the total platelet population.

MAO SA was determined using a modification of the method of Wurtman and Axelrod.¹⁷ ¹⁴C-Benzylamine (ICN, SA 4 Ci/mmole) and ¹⁴C-tryptamine (New England Nuclear, SA 10 Ci/mmole) were used as substrates at 0.5 mM. MAO SA were expressed as nanomoles of ¹⁴C-aldehyde formed per hour per milligram platelet particulate protein. Benzylamine was the preferred substrate for type B MAO, the predominant type in platelets,¹⁸ while tryptamine was a mixed type A and type B substrate.¹⁹

RESULTS

Patients with ATP are known to have low platelet counts and a high percentage of megathrombocytes. We present here the first report that these patients also were found to have the following differences when compared to the

Table 1. Specific Activity (SA) of MAO and Other Parameters of the Total Platelet Population of Normal Subjects and Patients With Autoimmune Thrombocytopenic Purpura (ATP) or Reactive Thrombocytosis (RT)

Parameter	Normal Subjects (n = 19)	ATP Subjects* (n = 6)	RT Subjects (n = 6)
MAO SA†			
Benzylamine (nmoles/hr/mg)	95.7 ± 25.9	45.8 ± 15.7‡	147 ± 43§
Tryptamine (nmoles/hr/mg)	10.1 ± 3.1	4.8 ± 3.3§	14.9 ± 3.1§
Benzylamine (nmoles × 10 ⁻⁹ /hr/platelet)	24.6 ± 14.6	6.0 ± 4.2‡	54.5 ± 26.8
Platelet count × 10 ³ /μl	269 ± 58	93 ± 47§	633 ± 101§
Percent megathrombocytes	13.6 ± 5.7	21.5 ± 7.4‡	5.1 ± 4.3§
Platelet volume (fl)	5.4 ± 0.7	6.4 ± 1.3	3.9 ± 0.9§
Platelet protein density (g × 10 ⁻¹⁵ /fl)	114 ± 70	48.8 ± 22.8	215 ± 95§
Platelet particulate protein density (g × 10 ⁻¹⁵ /fl)	49.4 ± 31	20.7 ± 8.9‡	90.6 ± 39.5§
Protein per platelet (g × 10 ⁻¹⁵)	591 ± 322	306 ± 134	855 ± 489
Particulate protein per platelet (g × 10 ⁻¹⁵)	257 ± 142	131 ± 61§	371 ± 213

All values are given as mean ± SD.

*Three subjects were splenectomized and were not significantly different from subjects with intact spleens.

†All SA reported were determined using platelet particulate protein.

‡Significantly different from normals at $p < 0.001$.

§ $p < 0.01$.

|| $p < 0.05$.

normal group: 1.9-fold lower mean protein per platelet; 2.3-fold lower platelet protein density, defined as platelet protein in grams × 10⁻¹⁵/unit volume platelets (mean platelet volume in femtoliters × platelet count in microliters); 2.0-fold lower particulate protein per platelet; and 2.4-fold lower platelet particulate protein density. The mean SA of MAO, using either benzylamine or tryptamine as substrate, was 2.1-fold lower than that of the normal group and 3.2-fold lower than that of the RT group. The mean SA of MAO per platelet using either substrate was also significantly lower than that of the normal group or RT group (Table 1).

Patients with RT, in addition to having high platelet counts and a reduced percentage of megathrombocytes, also had 1.5-fold higher mean protein per platelet when compared to the normal group, 1.9-fold higher platelet protein density, 1.4-fold higher particulate protein per platelet, and 1.8-fold higher platelet particulate protein density. Platelet protein density was 4.3-fold greater than in the ATP patients. In the RT group, the mean SA of MAO with either substrate was 1.5-fold higher than that of the normals and 3.2-fold higher than that of the ATP patients. The mean SA of MAO per platelet using benzylamine or tryptamine as substrate was also significantly higher than the normal or ATP group (Table 1).

DISCUSSION

The mechanisms governing the relationship of these platelet parameters to MAO are not known. The activity of an enzyme in a circulating platelet is probably determined by specific genetic factors regulating the formation of the enzyme during the megakaryocyte stage of platelet precursor life, as well as by genetic and environmental factors that regulate the rate of platelet production, the stage of development at release, and the mean age of the circulating platelet.²⁰⁻²⁴ It is believed that at least some of these factors also determine platelet number, percent megathrombocytes, platelet volume, and platelet weight.^{10,13,20,22,24-28}

In RT, the increased platelet number occurs in response to a physiologic stress. From our observations, it can be seen that this results in changes in the structural characteristics (platelet volume and protein density) of the platelets with an elevation of MAO activity possibly secondary to these structural changes. In ATP, the rapid clearance of antibody-coated platelets^{11,12} places an extreme demand upon platelet production, possibly leading to the premature release of platelets with decreased enzyme activity and decreased platelet protein density. In addition, the platelets that have been coated by antibody may possibly lose constituents, including MAO, prior to clearance, resulting in a more rapid loss of circulating platelet enzyme activity. In both conditions, the activity of MAO has probably been affected by the unusual demands on the normal mechanisms involved in regulating platelet production and clearance. This process results in a change in the structural and biochemical characteristics of the platelets in a given subject's platelet population.

There is evidence that a normal human subject has a platelet population which is heterogeneous for a number of characteristics, including platelet volume and platelet weight.^{15,20,21,23,25,26} It has been found that the mean specific activity of the enzymes of carbohydrate metabolism in platelets from a blood sample is the resultant of a wide range of activities in the individual platelets of that sample.²¹⁻²³

It seemed likely that an individual's platelets would also be heterogeneous for MAO. Accordingly, five different platelet density fractions were examined. Platelet MAO activity and protein content were found to be heterogeneously dispersed. There was a 15-fold greater enzyme activity and 18-fold greater protein content in the extreme heavy compared to the extreme light platelet fraction (Fig. 1). The highest percentage of both particulate and total platelet protein (not shown) was also associated with the heavy fractions, decreasing sharply in the lighter fractions. The present method of isolation of platelet fractions excluded those heavy-large platelets entrapped in the red blood cells and buffy coat following preparation of PRP. If this fraction had been included,²⁵ an even greater difference might have been obtained when the extreme heavy and light platelet fractions were compared. When data from this differential centrifugation study and data from the two aberrant groups are considered, it appears that high MAO activity is associated with heavy platelets densely packed with protein, rather than with large platelets per se.

The level of MAO (expressed per milligram protein) has been reported to be abnormally low in several mental conditions,¹⁻⁷ iron-deficiency anemia,²⁹ essen-

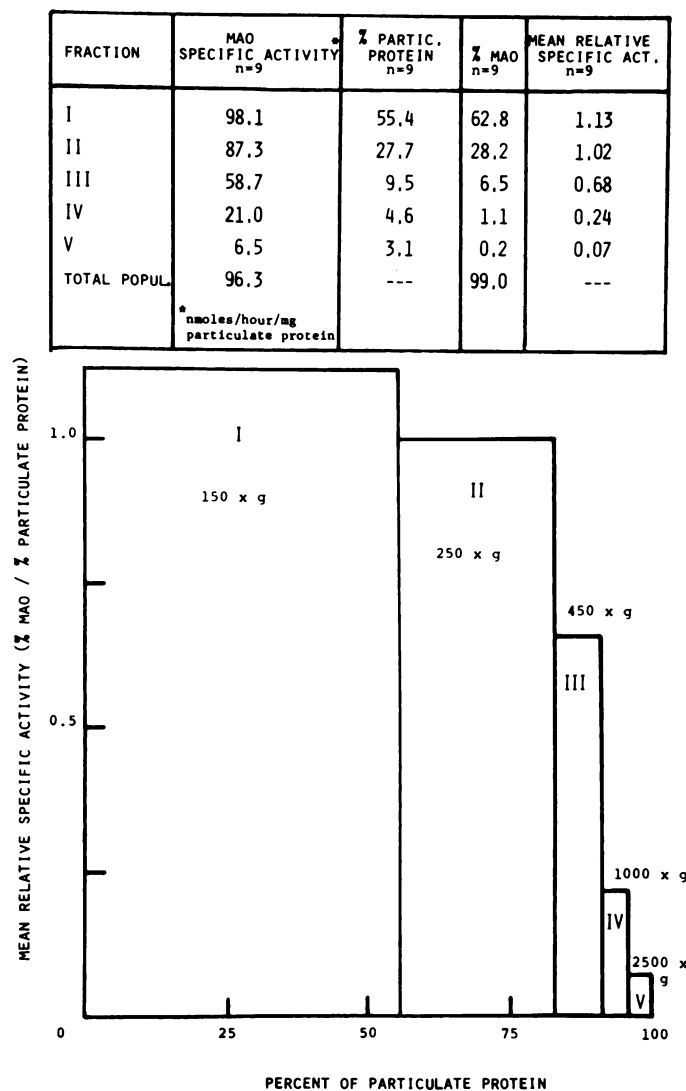


Fig. 1. MAO distribution in human blood platelets from normal subjects in histogram form according to de Duve.³⁷ Mean relative specific activity (percentage MAO activity, ¹⁴C-benzylamine as substrate, divided by the percentage platelet particulate protein) is plotted against the percentage platelet particulate protein inscribed cumulatively in order of the fractional platelet isolation (increasing sedimentation velocity). Percentage platelet particulate protein is defined as the platelet particulate protein content in each fraction divided by the total platelet particulate protein. The shape of the distribution curve using ¹⁴C-tryptamine (not shown) was identical.

tial hypertension and migraine,³⁰ and juvenile diabetes.³¹ Platelet MAO has been reported to be, in large measure, under genetic control.^{2,4} However, our results indicate that nongenetic factors such as platelet number, volume, and protein density should also be considered as possible determinants of platelet MAO activity in a sample of unfractionated platelets. Changes in these factors

could be induced by stress, epinephrine release,^{32,33} stages of the menstrual cycle,^{22,34} acute or chronic blood loss,^{22,34} drugs,³⁵ or iron deficiency,^{34,36} as well as other influences which might produce significant shifts in the distribution of a heterogeneous platelet population.

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