EVIDENCE FOR INTERACTING CATALYTIC SITES OF HUMAN PLATELET MONOAMINE OXIDASE

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SUMMARY: Studies with human platelet monoamine oxidase show that the enzyme contains two interacting catalytic sites. One site has a high affinity for phenylethylamine and its activity is noncompetitively inhibited by either benzylamine or tryptamine. However, benzylamine and tryptamine act as fully competitive inhibitors for each other and thus appear to be deaminated as a separate site. The K_m for each amine was in good agreement with the K_I determined when it was used as an inhibitor.

Human blood platelets previously have been found to contain monoamine oxidase (MAO: E.C. 1.4.3.4.) activity (1,2). The enzyme has been solubilized by sonication in the presence of Triton X-100, and has been found to have an estimated molecular weight of 235,000. Furthermore, it appears to be electrophoretically homogeneous, in contrast to MAO in other tissues (3). Platelet MAO is relatively specific for the substrates phenylethylamine and benzylamine and is sensitive to inhibition by deprenyl but not by clorgyline; these characteristics indicate that the platelet enzyme is similar to the type B MAO described in other tissues (4).

We have recently demonstrated that tricyclic antidepressant drugs inhibit *in vitro* human platelet MAO (5). The mechanism of inhibition was found to be dependent upon the substrate used. Thus, amitriptyline exhibited either noncompetitive or competitive inhibition when the substrates phenylethylamine and benzylamine were used, respectively. This suggested that the platelet contains either multiple enzyme forms or that the enzyme has multiple catalytic sites. The present study provides evidence for the latter possibility and indicates that one active site has a high affinity for phenylethylamine, while

the other has a high affinity for both benzylamine and tryptamine.

MATERIALS AND METHODS: MAO was assayed radiochemically as described previously (5) using the procedure of Wurtman and Axelrod (6) with the substrate [2-^{14}C]tryptamine (New England Nuclear), [1-^{14}C]phenylethylamine (New England Nuclear) and [7-^{14}C]benzylamine (Mallinkrodt). Unlabeled substrates were obtained as the hydrochloride salts from Aldrich Chemical Co. (tryptamine) and K & K Laboratories (benzylamine and phenylethylamine). Platelets were isolated as previously described (5). The kinetic studies below were performed using either platelet sonicates or partially pruified enzyme, as indicated. Partially purified MAO was prepared by solubilization in Triton X-100 and precipitation by 30-55% saturation of ammonium sulfate, according to the procedure of Collins and Sandler (3). Partially purified enzyme was subjected to polyacrylamide gel electrophoresis in .05 M Tris buffer (pH 8.6), using a current of 5 mA/tube for 2 hr. MAO activity in the gel for each substrate was located either by staining with nitroblue tetrazolium (3) or by assaying gel slices radiochemically. Gel slices (2mm) were allowed to soak overnight (4°) in 1 ml 0.2M phosphate buffer, and then homogenized before assaying. Data were plotted according to Lineweaver-Burk (7) using least mean squares.

RESULTS: Gel electrophoresis of solubilized, partially purified platelet MAO resulted in enzymatic activity in two distinct bands, when the activity was measured either by tetrazolium staining (Figure 1) or by radiochemical assay of gel slices. One band of activity appeared to coincide with a visible yellow band, which was presumably due to the flavin content of the enzyme. Another band of activity normally was found at the top of the gel and thus appeared to contain a high molecular weight material. If electrophoresis was carried out for an initial one-half hour at 2mA/tube and then for 2 hr. at 5mA/tube, the slower band of activity migrated a few millimeters into the gel. The proportion of the activity in the slower band was dependent upon the amount of enzyme layered on the gel, suggesting that the activity contained

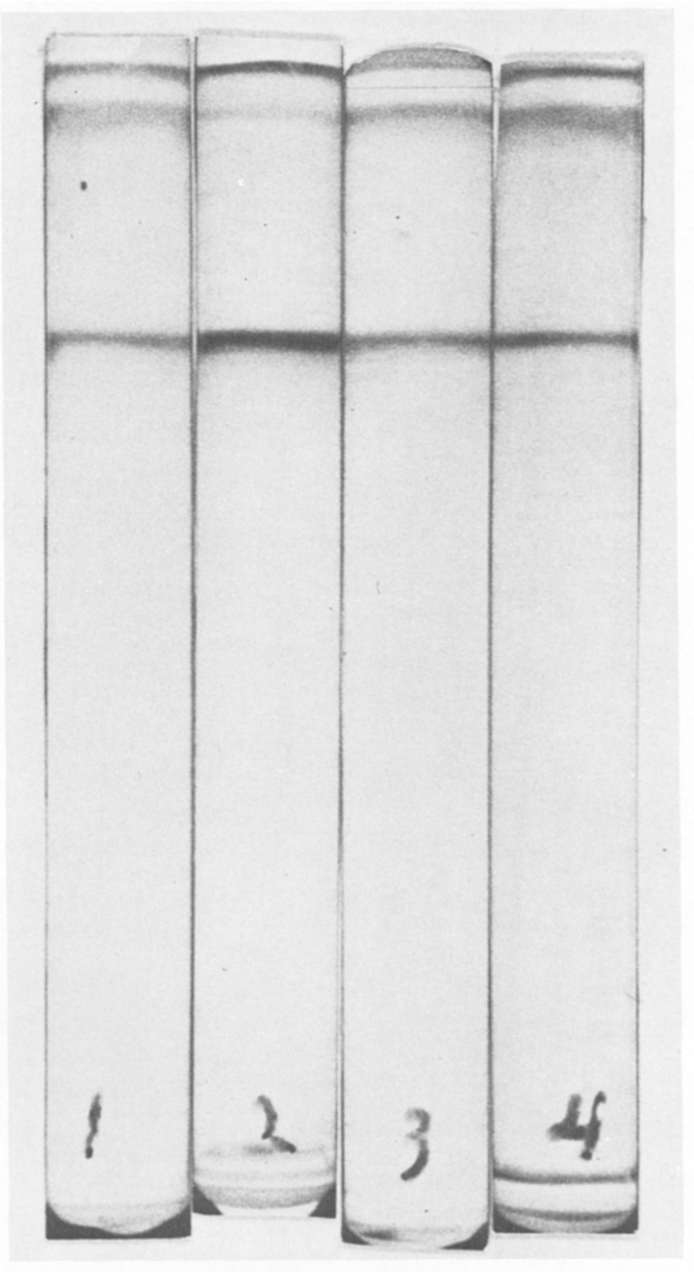

Figure 1. Gel electrophoresis of solubilized, partially purified platelet MAO. Enzymatic activity was visualized by tetrazolium staining in the presence of the following substrates: 1. phenylethylamine; 2. tryptamine; 3. benzylamine; 4. all three.

in the large molecular weight material was due to protein aggregation. Moreover, each of the two bands had the same relative activity for each of the substrates used. Thus, from these data we have found no evidence for isoenzymes of platelet MAO specific for different substrates.

In order to determine whether platelet MAO might contain different catalytic sites for different substrates, enzymatic activity was determined for each of the three [^{14}C]-labeled substrates in the presence of unlabeled, varying concentrations of each of the other two amines. Thus, six separate kinetic experiments were performed, three of which are illustrated in Figures 2-4. Although the data for these figures were obtained using an enzyme preparation which had been solubilized with Triton X-100 and isolated by ammonium sulfate fractionation, identical results were obtained when whole platelet sonicates were used as an enzyme source.

Figure 2 shows that unlabeled tryptamine competitively inhibits the MAO activity measured with [^{14}C]-benzylamine. A plot of 1/v versus i gave a

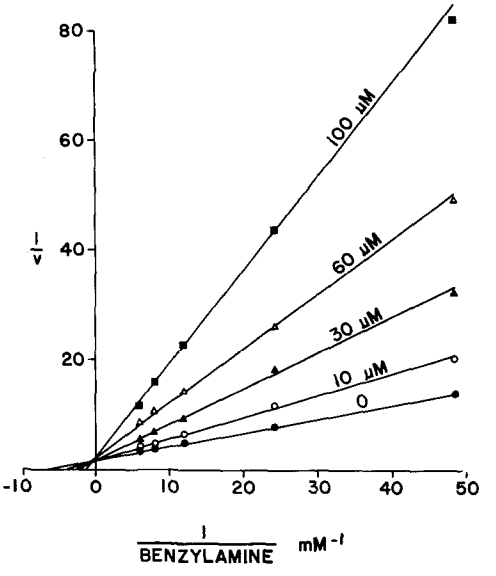

Figure 2. Inhibition by tryptamine of partially purified platelet MAO using the substrate benzylamine.

linear relationship, indicating that the inhibition is fully competitive (8). The converse experiment (i.e., benzylamine was used as the inhibitor for MAO activity measured with the substrate [^{14}C]-tryptamine) also resulted in competitive inhibition. Thus, these results indicate that tryptamine and benzylamine act as substrate analogues and bind at a common active site.

Figure 3 illustrates that the MAO activity determined with [^{14}C]-tryptamine as substrate was inhibited by phenylethylamine in a mixed non-competitive manner. Moreover, MAO activity determined with [^{14}C]-phenylethylamine as substrate was inhibited noncompetitively by benzylamine (Figure 4). This inhibition appeared to become a mixed type at high concentrations of benzylamine. The converse experiments of those shown in Figures 3 and 4 also resulted in mixed noncompetitive and noncompetitive inhibition, respectively. These data demonstrate that phenylethylamine (as either a substrate or as an inhibitor) binds to platelet MAO noncompetitively with respect to tryptamine or benzylamine, suggesting that phenylethylamine is

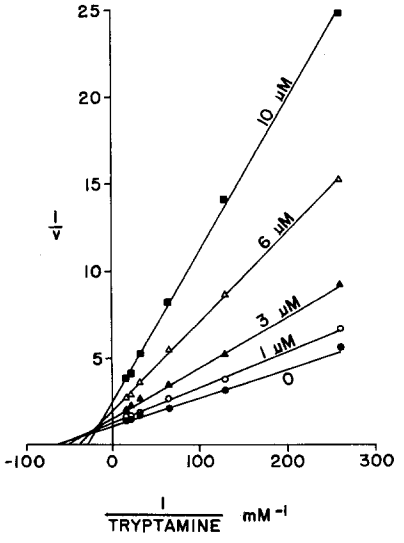

Figure 3. Inhibition by phenylethylamine of partially purified platelet MAO using the substrate tryptamine.

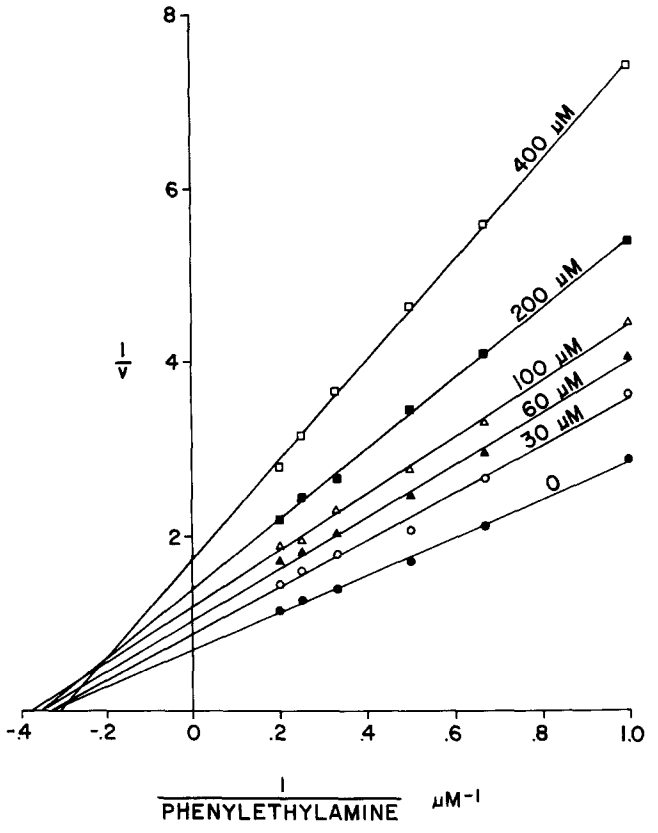

Figure 4. Inhibition by benzylamine of partially purified platelet MAO using the substrate phenylethylamine.

deaminated as a separate interacting catalytic site.

Table 1 shows that the binding affinities for each of the three amines were approximately the same whether the binding constant was determined for that amine as either a substrate (measured as K_m) or as an inhibitor for the oxidation of one of the other amines (measured as KI_s). For example, the K_m for tryptamine was found to be 17 and 19 µM in two experiments; this is in agreement with the inhibitory constant measured for tryptamine, 22 and 17 µM, when either phenylethylamine or benzylamine, respectively, were used as substrates.

Table 1

Binding Affinities of Phenylethylamine, Tryptamine, and Benzylamine Determined as Substrates (K_m) and Inhibitors (K_{I_s})

Amine	K_m (μM)	K_{I_s} (μM)	Substrate used for determination of K_{I_s}
Phenylethylamine	3.7,3.0	1.9	Tryptamine
		1.1	Benzylamine
Tryptamine	17,19	22	Phenylethylamine
		17	Benzylamine
Benzylamine	157,145	184	Phenylethylamine
		170	Tryptamine

DISCUSSION: These studies demonstrate that human platelet MAO contains separate, interacting catalytic sites for phenylethylamine and for benzylamine and tryptamine. That these enzymatic activities could not be separated by gel electrophoresis is consistent with the previous finding that platelet MAO was electrophoretically homogeneous (3). On the other hand, these data appear to agree with the finding that tricyclic antidepressant drugs showed differential effects on platelet MAO when different substrates were used (5).

The existence of separate interacting catalytic sites specific for different aromatic amines suggests a possible mechanism for the control of the metabolism of these amines in human platelets. A similar regulatory role was suggested by the finding that serotonin and norepinephrine exhibit noncompetitive kinetics toward each other for MAO activity of synaptosomal mitochondria in bovine thalamus and hypothalamus (9). A possible regulatory

role for iodotyramine has been suggested by its noncompetitive inhibition of bovine thyroid microsomal MAO when tyramine was used as substrate (10). Since this enzyme was found to deaminate phenylethylamine but not tryptamine or benzylamine, its catalytic properties would appear to be markedly similar to those of the phenylethylamine-specific site of the human platelet enzyme.

Since the human platelet contains only MAO which is characteristic of the type B form, the platelet would appear useful in further studies to elucidate the characteristics and functions of type B MAO in brain and other tissues. It also would be important to determine whether the activity of the platelet enzyme reflects that of a particular form of brain MAO, although platelet MAO activity recently has been used as a biochemical correlate of schizophrenia and depression (4).

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