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MOLECULAR WEIGHT DIFFERENCES BETWEEN HUMAN PLATELET AND PLACENTAL MONOAMINE OXIDASE

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Abstract—The molecular weights of the active site subunits of human placental and platelet monoamine oxidase have been compared. These tissues were chosen as each appears to contain only one form of the enzyme, type A in placenta and type B in platelets. We found that the biochemical properties (substrate affinity and inhibitor sensitivity) clearly distinguish between the enzymes from these two sources, whether in the membrane-bound or detergent-solubilized state. The suggestion that such differences result from the existence of distinct molecular forms was strengthened by the observation that the active site subunits labelled specifically with [^{3}H]pargyline differ in their apparent molecular weights and in the electrophoretic pattern of their partial proteolytic digest products.

Monoamine oxidase catalyses reactions of the form:

$$R - CH_2 - NH_2 + H_2O + O_2 = R - CHO + NH_3 + H_2O_2.$$

A large number of naturally occurring and synthetic amines can act as substrate [1] and the enzyme is widely distributed [2]. In mammalian cells, the enzyme is located on the outer mitochondrial membrane [3] and contains covalently bound flavin adenine dinucleotide as a prosthetic group [4].

Early studies of the activity of the enzyme towards different substrates showed considerable variation between different tissues of the same species [5]. The discovery of several irreversible inhibitors of the enzyme, coupled with these variations in substrate affinity, permitted a classification of the enzyme into two forms, types A and B [6–8]. The type A enzyme is selectively inhibited by clorgyline and is preferentially active towards 5-hydroxytryptamine, whereas the B type is more active towards phenylethylamine and benzylamine and is selectively inhibited by deprenil.

The relationship between the two enzymes as distinguished by these criteria is at present unclear, although it has been suggested that there may be a single protein species whose activity is modified by the lipid environment of the mitochondrial membrane in different tissues [9, 10].

Whereas earlier studies indicated that solubilization of the enzyme from tissues possessing both forms resulted in either alteration in their properties [11] or selective inactivation of one of the forms [12], it is now becoming apparent that monoamine oxidases can be solubilized from the outer mitochondrial membrane without alteration of their biochemical properties [13].

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In the present study, we have compared the monoamine oxidases from human platelets and placentae. These tissues were chosen on the basis of preliminary studies which indicated that the enzymes from the two sources possessed significantly different inhibitor and substrate specificities. Each of these tissues appears to contain predominantly one form of monoamine oxidase which satisfies the criteria for either type A (placenta) or type B (platelet) and both can be obtained in soluble forms which retain the general properties of the native enzymes.

Examination of the apparent molecular weights of the active site subunits, which had been labelled with [^{3}H]pargyline, indicates that the placental form differs significantly from the platelet form, thereby providing direct evidence that the different biochemical properties of the enzyme in various tissues may reflect underlying differences in the primary structure of the protein. This observation is complemented by observations to be presented in a subsequent report that placental and platelet forms of the enzyme possess different immunological determinants.

MATERIALS AND METHODS

Materials [G -^{3}H]tryptamine hydrochloride and 5-hydroxy[G - ^{3}H]tryptamine creatinine sulphate complex were obtained from the Radiochemical Centre, Amersham, U.K.; B-[ethyl-1-^{14}C]phenylethylamine and [benzylmethylene-^{3}H]pargyline hydrochloride were obtained from New England Nuclear, Boston, MA, U.S.A.

Tryptamine hydrochloride, phenylethylamine, 5-hydroxytryptamine–creatinine sulphate complex, di(2-ethylhexyl) phosphoric acid, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES), *N'*,*N*-methylene bisacrylamide, pargyline and Triton X-100 were purchased from Sigma Chemical Co, St.

Louis, MO, U.S.A. Clorgyline was a kind gift from Dr. A. Crichton, May & Baker Ltd., Dagenham, U.K. DEAE cellulose was obtained from Whatman Ltd., Maidstone, U.K. Sepharose 6BCL, Ficoll 400 and Dextran 500 from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A., and polyethylene glycol 6000, sodium lauryl sulphate (specially pure), *N,N'*-diallyltartardiamide and *NNN'N'*-tetramethylethylene diamine from BDH Chemicals Ltd. Protease V (*Staphylococcus aureus*) was purchased from Mile Biochemicals, Slough, U.K. All other materials were obtained from Fisons Ltd., Loughborough, U.K. and were of analytical reagent grade.

Monoamine oxidase assays. A radiochemical assay was used with [^{3}H]tryptamine, [^{3}H]5-hydroxytryptamine and [^{14}C]phenylethylamine as substrates based on the method of Wu and Dyck [14] with a single modification. It was found that the efficiency of the step in which a liquid ion exchange resin is employed to bind unreacted substrate could be improved by increasing the concentration of the di(ethylhexyl) phosphoric acid to 1.25 M and adding this in a very small volume (20 μl = 1/10 the reaction volume), thus minimizing the partitioning of neutral reaction products between the aqueous and chloroform phases.

The standard assay used [^{3}H]tryptamine as substrate as it is readily oxidized by both types of monoamine oxidase [6]. Final concentrations were 1 mM (1.5 nCi/nmole) for the placental enzyme and 80 μM (0.1 μCi/nmole) for the platelet enzyme. These concentrations were chosen after the apparent K_m values had been determined for each enzyme by assay over a wide range of substrate concentrations (0.2–5 × K_m). K_m values (10 μM for the platelet enzyme and 200 μM for the placental enzyme) were then obtained by statistical treatment of the data, as described by Wilkinson [15]. The concentrations above are both 5 times the K_m concentrations and will therefore measure 90 per cent of the apparent maximal velocity obtainable in the system used.

The assay was linear with respect to time and enzyme concentration for all substrates and the product separation system gave reliable and reproducible values, allowing comparisons of relative reaction rates, although absolute values could not be obtained due to difference in the efficiency of extraction of the different products. The reactions were performed in 20 mM HEPES/KOH, pH 8.0, in a total volume of 0.2 ml. The assays were usually carried out over 30 min at 37°. Enzyme activity is expressed as nmoles. mg protein^{-1}. min^{-1} and protein concentrations were determined by the method of Lowry *et al.* [16].

Purification procedures. Placental and platelet monoamine oxidases were solubilized and partially purified by a method based on procedures developed for the bovine brain enzyme by McCauley and Racker [17]. However, it was observed that both platelet and placental enzymes were inactivated by ammonium sulphate fractionation so this step was omitted. As an alternative, for the platelet enzyme, a phase separation method, similar to that used by Salach [18] and based on a method of Albertsson [19], was developed.

Placental monoamine oxidase. Human placentae were obtained immediately after delivery. They were dissected free of the cord and membranes and cut into small pieces. All subsequent operations were carried out at 4°.

The pieces were washed in isolation medium (0.1 M Tris, 0.25 M sucrose and 0.02 M EDTA, pH 7.2) and suspended in 2 vol. of the same solution. The tissue was homogenized in a Silverson homogenizer, filtered through gauze and centrifuged at 1000 *g* for 20 min.

The supernatant fraction was centrifuged at 23,000 *g* for 30 min and the pellet washed with 2 vol. of isolation medium with centrifugation at 38,000 *g* for 20 min. The mitochondria so obtained were resuspended to a final concentration of 20mg protein/ml in 50 mM potassium phosphate, pH 8.0.

An aliquot of the placental mitochondria was brought to 0.5% w/v with respect to Triton X-100, stirred for 30 min, and centrifuged at 70,000 *g* for 30 min. The pellet was resuspended in 50 mM potassium phosphate, pH 8.0, containing 1% w/v Triton X-100 and sonicated for 10 min on ice in an M.S.E. Sonicator (large probe, maximum amplitude). The suspension was centrifuged at 70,000 *g* for 45 min and the supernatant fraction dialysed against 10 mM potassium, pH 8.0, for 24 hr.

The dialysed sample was applied to a column of DEAE cellulose pre-equilibrated with 10 mM potassium phosphate, pH 8.0, and the column washed with two bed volumes each of 10 and 100 mM potassium phosphate, pH 8.0. The enzyme was then eluted with a linear gradient of 0–1.0% w/v Triton X-100 in 100 mM potassium phosphate, pH 8.0.

The fractions containing the peaks of monoamine oxidase activity were pooled and concentrated in a Sartorius Membranfilter SM 13200 collodion bag (Sartorius Membranfilter GmbH). They were then applied to a column of Sepharose 6BCL and the enzyme eluted with 50 mM potassium phosphate, pH 8.0.

The fractions containing monoamine oxidase activity were pooled and concentrated as above. The enzyme was stored at −70° at a protein concentration of 1.0 mg/ml.

Platelet monoamine oxidase. Platelet-rich plasma was obtained from the Oxford Blood Transfusion Service. The platelets were collected by centrifugation at 6000 *g* for 30 min. They were washed once in 50 mM potassium phosphate, pH 8.0, resuspended in the same buffer and sonicated for 1 min on ice. All subsequent operations were carried out at 4°.

The suspension was centrifuged at 38,000 *g* for 1 hr and the pellet resuspended in 50 mM potassium phosphate, pH 8.0, containing 1.0% w/v Triton X-100 and sonicated for 5 min. on ice. After centrifugation at 100,000 *g* for 1 hr, the supernatant fraction was mixed with Dextran 500, Ficoll 400 and polyethylene glycol 6000 to final concentrations of 6.67% w/v, 8% w/v and 5.33% w/v, respectively. The mixture was then centrifuged at 30,000 *g* for 45 min in a swing-out rotor and the Ficoll layer collected. This was dialysed against 10 mM potassium phosphate, pH 8.0, for 24 hr before being applied to a DEAE cellulose column preequilibrated with 10 mM potassium phosphate, pH 8.0. Subsequent operations were the same as for the placental enzyme.

Separation of different molecular weight forms

Interconversions of different molecular weight forms of solubilized platelet and placental monoamine oxidases were studied by density gradient centrifugation with either glycerol or Ficoll as the dense medium. The choice of these compounds was determined by the stability of the particular enzyme type under the centrifugation conditions used. The placental enzyme was quite stable in glycerol solutions between 10 and 30% v/v, whereas the platelet enzyme was more stable in Ficoll solutions of 7.5–20% w/v. These ranges of glycerol and Ficoll concentrations are equivalent with respect to density.

Linear gradients of glycerol or Ficoll 400 in 50 mM potassium phosphate, pH 8.0, over the above ranges were poured, the enzyme samples layered on the surface and the tubes centrifuged at 90,000 *g* for 14 hr. Fractions obtained from these gradients were then assayed for monoamine oxidase activity.

[^{3}H]Pargyline binding

Pargyline inhibition of platelet and placental monoamine oxidases in both membrane-bound and soluble forms was determined by assay of residual enzyme activity after 20 min preincubation at 37° with pargyline concentrations ranging from 10^{-3} to 10^{-10} M. pI_{50} values obtained were 10^{-8} M for the platelet enzyme and 10^{-6} M for the placental enzyme. The values were the same for membrane-bound or soluble enzyme and in all cases a simple sigmoid dose–response curve was obtained with tryptamine as substrate.

Conditions were established whereby the enzymes could be labelled with [^{3}H]pargyline to a constant specific activity which was associated with complete loss of monoamine oxidase activity. This was achieved by 60 min incubation at 37° with either 4 μM [^{3}H]pargyline (placental enzyme) or 1 μM [^{3}H]pargyline (platelet enzyme). The specificity of labelling was established by showing that it could be prevented by preincubation with appropriate concentrations of clorgyline or tryptamine. The [^{3}H]pargyline used had a specific activity of 6.86 Ci/mmole and labelled preparations of specific activity ~2 μCi/mg protein were obtained. The labelled enzymes were dialysed to constant specific activity against 50 mM potassium phosphate, pH 8.0.

Polyacrylamide gel electrophoresis

The method of O'Farrell [20] was used for the electrophoresis of [^{3}H]pargyline-labelled monoamine oxidase preparations in sodium dodecyl sulphate-containing polyacrylamide gels. A Bio-Rad vertical slab gel apparatus of dimensions 15 × 12 × 0.15 cm was used: Slab gels were fixed overnight in 50% trichloroacetic acid and stained for protein with a solution of 0.1% Coomassie Brilliant Blue in 5% (v/v) methanol and 10% (v/v) acetic acid and destained with 10% (v/v) acetic acid. Gels were then prepared for fluorography by the method of Bonner and Laskey [21] and exposed to Fuji RX medical X-ray film at −70° for 1–2 weeks.

The [^{3}H]pargyline-labelled enzymes were also studied by several additional electrophoretic techniques. The possibility of anomalous migration (e.g. due to the presence of carbohydrate residues) was studied using sodium dodecylsulphate-containing polyacrylamide gels which were formed with a linear horizontal gradient of acrylamide concentration from 5–10% following the method of Beckendorf and Kafatos [22]. A mixture of the two enzymes, labelled with [^{3}H]pargyline, was electrophoresed in this system, then processed as above.

Similar properties were investigated by using a crosslinking agent, *N,N'*-diallyltartardiamide, which interacts with glycosylated proteins, retarding their migration as described by Heine *et al.* [23].

A preliminary study of the peptide structure of the platelet and placental monoamine oxidase active site subunits was undertaken by partial proteolytic digestion of the labelled enzymes in sodium dodecylsulphate following the method of Cleveland *et al.* [24]. [^{3}H]Pargyline-labelled, partially purified preparations were incubated with varying concentrations of Protease V (*S. aureus*) for 30 min at 37° in the proteolytic digestion buffer of Cleveland *et al.* [24]. After boiling for 1 min, the proteolytic fragments were then resolved on a sodium dodecylsulphate – 15% polyacrylamide gel and further processed as above.

RESULTS

The aim of this work was to compare monoamine oxidases from two human tissues each of which appear to express only one form of the enzyme. This was done for two reasons. First, to see if differences in biochemical properties were associated with differences in physical structure, and second, to characterize the enzymes sufficiently to facilitate subsequent immunological studies. The properties of the two enzymes were compared during various stages of solubilization and purification (Table 1). One feature of note is the large difference in the level of activity of enzyme in the two tissues, a difference which persists throughout the various procedures. Examination of the relative turnover numbers of the two enzymes using [^{3}H]pargyline to estimate the active site concentration indicated that the differences in activity could be largely accounted for by the differences in relative turnover numbers (5 molecules/min for the platelet enzyme and 220 molecules/min for the placental enzyme).

After solubilization, it was found that the detergent complexes of both types of monoamine oxidase existed in two distinct molecular weight forms. On Sepharose 6BCL, each enzyme eluted in two peaks, one near the void volume (peak I) and one partly included in the gel (peak II). However, the elution volumes for both forms of the platelet enzyme were greater than those for the corresponding placental forms. Approximate molecular weights were determined as follows: peak I placental 2×10^6, peak II placental 9×10^5, peak I platelet 1.2×10^6, and peak II platelet 6×10^5. These peaks were each separately analysed by rechromatography on Sepharose 6BCL and found to elute as single peaks.

The interconversion of the different molecular weight forms was studied by density gradient centrifugation on glycerol and Ficoll 400. Figure 1 shows the profile of placental monoamine oxidase on a 10–

Table 1. Activity at various stages of purification of placental and platelet monoamine oxidases

	*Specific activity (nmoles·mg protein^{-1}·min^{-1})	Units (nmoles/min)	Yield (%)	Purification (fold)
Placental enzyme				
Stage:				
Mitochondria	2.3	3062	100	–
Triton X-100 extract	34.6	2768	90	15
DEAE cellulose chromatography	73.9	2204	72	32
Platelet enzyme				
Stage:				
Sonicated platelets	0.02	27	100	–
Triton X-100 extract	0.07	17	63	3.3
Phase separation/DEAE cellulose chromatography	0.29	11	41	14.5

* Tryptamine as substrate.

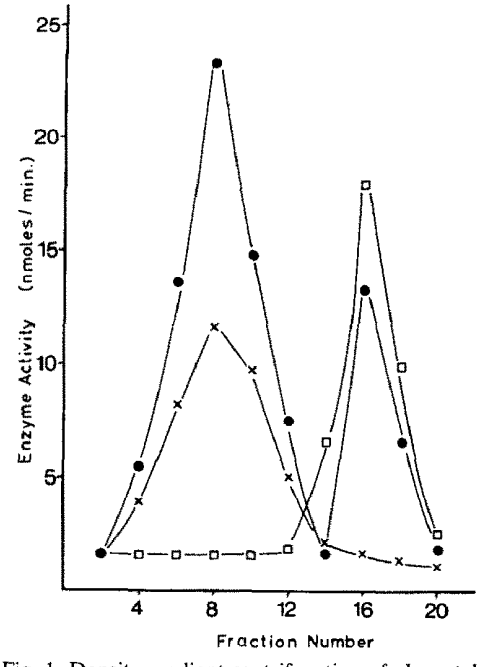

Fig. 1. Density gradient centrifugation of placental monoamine oxidase. Profile of placental monoamine oxidase activity after centrifugation for 14 hr at 90,000 *g* in a 10–30% v/v linear gradient of glycerol in 50 mM potassium phosphate, pH 8.0. A swing out rotor of 23 ml capacity was used and 1 ml fractions were collected. The activity of alternate fractions is shown for three samples: (1) a mixture of peaks I and II obtained from Sepharose 6BCL chromatography (●—●); (2) an aliquot of the peak I form incubated for 4 hr at 4° with 0.1% w/v Triton X-100 (□—□); and (3) an aliquot of the peak II form dialysed extensively against 50 mM potassium phosphate, pH 8.0, without Triton X-100 (×-×). Fractions of lower numbers are at the bottom of the gradient and the peaks of activity in fractions 8 and 16 correspond to the peaks I and II demonstrated on Sepharose chromatography (see Results). The density gradient for the sample of the high molecular weight form pretreated with Triton X-100 (□—□) contained 0.1% w/v Triton X-100.

30% v/v linear gradient of glycerol in the absence of Triton X-100. Two peaks of activity were observed and these were shown to be identical with the peaks I and II described above by Sepharose 6BCL chromatography. If samples of the isolated peak I of the placental enzyme were incubated for 4 hr at 4° with 0.1% w/v Triton X-100 before glycerol gradient centrifugation (in the presence of Triton X-100), all of the activity was located at the top of the gradient in the position of the low molecular weight (peak II) form. Conversely, if samples of the isolated peak II of the placental enzyme were dialysed extensively against 50 mM potassium phosphate, pH 8.0, before being subjected to glycerol density gradient centrifugation in the absence of Triton X-100, all of the activity was recovered at the bottom of the gradient in the position corresponding to that of the peak I form. Recovery of activity in these experiments was between 90 and 100 per cent, indicating quantitative interconversion of the different forms of the enzyme. Similar quantitative interconversion could be demonstrated if Sepharose 6BCL chromatography was employed to separate the different molecular weight forms rather than density gradient centrifugation.

Biochemical characterization

Sensitivity of the various enzyme preparations to the inhibitor clorgyline was studied using tryptamine, a substrate equally well oxidized by both forms of monoamine oxidase [7]. Dose–response curves for clorgyline inhibition of both membrane-bound and solubilized, partially purified monoamine oxidase from placentae and platelets are shown in Fig. 2. The inhibition curves for the solubilized preparations are presented separately for the different molecular weight forms. All forms show a single sigmoid dose–response curve but the pI_{50} values are quite different. Typical A-type behaviour is demonstrated by placental mitochondria and the higher molecular weight aggregate of solubilized placental enzyme (pI_{50} value 8.4). Similarly, membrane-bound platelet enzyme has a similar sensitivity to clorgyline inhibition as its corresponding high molecular weight form after

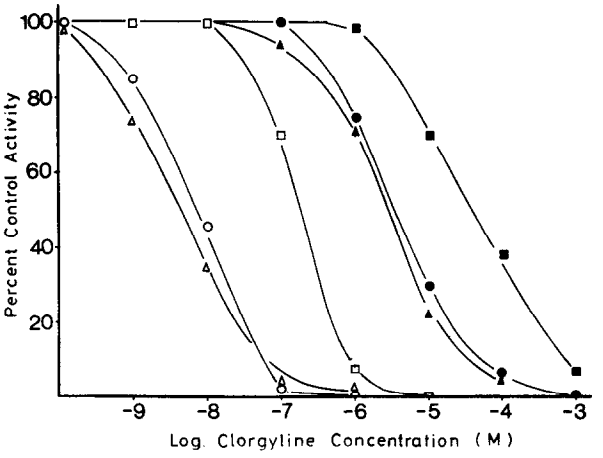

Fig 2. Clorgyline inhibition of different molecular weight forms of human placental and platelet monoamine oxidase. Dose–response curves for the irreversible inhibitor, clorgyline, are shown for: placental mitochondria (△—△), sonicated platelets (▲—▲) peak I, placental enzyme (○—○), peak II, placental enzyme (□—□) peak I, platelet enzyme (●—●) and peak II, platelet enzyme (■—■). Enzyme samples were pre-incubated with the inhibitor at the concentrations shown for 20 min at 37° before assay. The substrate used was tryptamine (1 mM, 0.002 μCi/nmole for the placental enzyme and 80 μM, 0.2 μCi/nmole for the platelet enzyme).

solubilization, but the pI_{50} of 4.3 indicates that this enzyme is type B.

Both lower molecular weight forms show significantly different pI_{50} values for clorgyline inhibition and in both cases, the shift is in the direction of reduced sensitivity. However, there is no overlap between the two forms.

In no case was any evidence found for an intermediate plateau in the dose–response curves and control incubations indicated that all forms were stable to incubation at 37° for up to 90 min. This is significantly longer than the times used for the inhibition studies so it is unlikely that the preparations used contained different forms of the enzymes which were preferentially inactivated non-specifically.

The patterns of sensitivity to clorgyline inhibition were also reflected in substrate specificities. Three substrates were used: 5-hydroxytryptamine (A type), tryptamine (A and B types) and phenylethylamine (B type). Relative reaction rates were calculated for each form based on its activity towards tryptamine. This overcomes any problem regarding differences in extraction of the products of the various substrates and enables comparisons to be made between the enzymes in their various forms. These relative reaction rates are presented in Table 2. All reaction rates were based on substrate concentrations of $5 \times K_m$ values which were determined individually for each enzyme form and appropriate substrate as described for tryptamine in Materials and Methods.

It can be seen that the placental enzyme forms all

Table 2. Reaction rates of various forms of placental and platelet monoamine oxidases relative to the rate measured with tryptamine as substrate*

	Relative reaction rate		
Enzyme	5-Hydroxytryptamine	Tryptamine	Phenylethylamine
Membrane bound placental	1.77	1	0.38
Solubilized placental			
(a) high mol. wt form	2.34	1	0.57
(b) lower mol. wt form	0.59	1	0.47
Membrane-bound platelet	0.20	1	1.20
Solubilized platelet			
(a) high mol. wt form	0.13	1	1.13
(b) lower mol. wt form	0.18	1	3.59

* Rates were determined at substrate concentrations of $5 \times K_m$. For details see text.

react preferentially with 5-hydroxytryptamine and at a low rate with phenylethylamine, whereas the platelet enzymes are more reactive towards phenylethylamine and have very low activity towards 5-hydroxytryptamine. For both tissues, the membrane-bound enzymes have similar properties to the higher molecular weight aggregates of the solubilized enzymes, in agreement with the results of clorgyline inhibition studies. The substrate affinities of the two lower molecular weight aggregates apparently reflect their relatively lower sensitivity to clorgyline inhibition.

Polyacrylamide gel electrophoresis

Solubilized samples of platelet and placental enzymes, labelled with [^{3}H]pargyline as described in Materials and Methods, were dissociated by heating to 100° for 2 min in the presence of 2% (w/v) sodium dodecylsulphate and 5% (v/v) β-mercaptoethanol

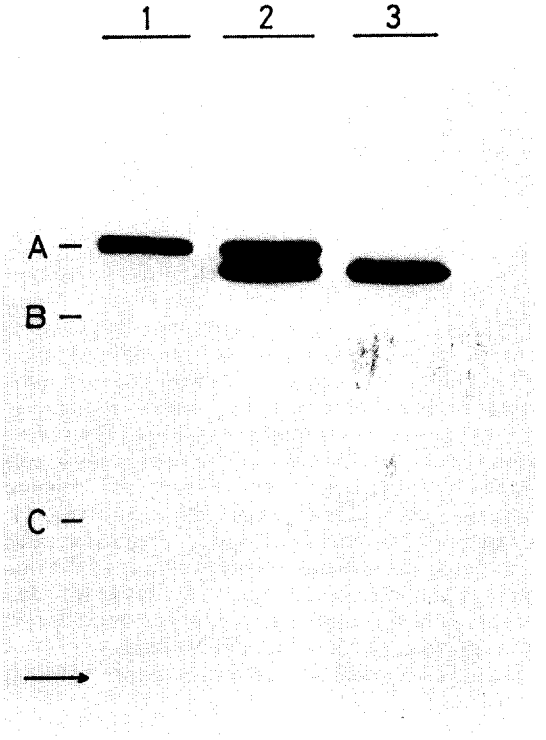

Fig. 3. Fluorograph of sodium dodecylsulphate–polyacrylamide gel of [^{3}H]pargyline-labelled monoamine oxidases. Preparation of platelet and placental monoamine oxidase were labelled with [^{3}H]pargyline as described in the text. They were then subjected to sodium dodecylsulphate–polyacrylamide gel electrophoresis (7% gel) following the method of O'Farrell [20]. After electrophoresis, the labelled bands were visualized by fluorography using the method of Bonner and Laskey [21]. Results are shown for samples of placental (track 1) and platelet (track 3) monoamine oxidase and a mixture of the two (track 2). Equal quantities of each enzyme (in terms of [^{3}H]pargyline counts) were run in each track. Molecular weights were determined by comparison with the mobility of standard proteins, bovine serum albumin (67,000), glutamate dehydrogenase (57,000) and lactate dehydrogenase (35,000), the positions of which (labelled A, B and C, respectively) were determined by protein staining of the gel prior to fluorography. The arrow indicates the position of the dye front.

and subjected to electrophoresis in polyacrylamide gels containing 0.1% (w/v) of the same detergent. It was observed that, under these conditions, the labelled subunits in the placental and platelet preparations were clearly resolved (see Fig. 3). Apparent molecular weights of the labelled components of about 67,000 daltons for the placental enzyme and 63,000 for the platelet enzyme were determined from a comparison with the observed mobilities of proteins of known molecular weight. A single band of labelled material was observed for each preparation at all stages of the purification and the molecular weight difference was consistently apparent.

It has previously been suggested that monoamine oxidase is a glycoprotein [12], in which case, anomalous migration on polyacrylamide gels due to the presence of carbohydrate residues might account for the differences in molecular weights described above [25]. This was studied by comparing mobility of the labelled preparations on polyacrylamide gels with varying acrylamide concentrations. Glycosylated proteins should give anomalously high apparent molecular weights at low acrylamide concentrations and a lower apparent molecular weight at higher concentrations [25]. If the degree of glycosylation differs, variation in the pattern of mobility change with changing acrylamide concentration might be expected [22]. However, similar mobility change profiles were obtained with mixtures of labelled placental and platelet monoamine oxidase on linear, horizontal 5–10% gradient polyacrylamide gels, suggesting that glycosylation is unlikely to be responsible for the observed differences in molecular weights between the two preparations (Fig. 4).

This interpratation is also supported by the observation that similar mobility changes were observed for both labelled enzyme preparations when these experiments were repeated with *N,N'*-diallyltartardiamide as cross-linker, an agent which interacts with glycoproteins and retards their migration [23].

Further evidence of differences in the structure of the [^{3}H]pargyline-labelled subunits of platelet and placental enzyme was obtained by comparing the electrophoretic pattern of the products resulting from their partial proteolytic digestion. Incubation of equivalent amounts of enzyme from the two sources (adjusted on the basis of [^{3}H]pargyline binding) with increasing amounts of protease V resulted in several fragments. Although some of these were of similar apparent molecular weights, the electrophoretic patterns were clearly distinguishable (see Fig. 5). One of the most distinctive features was the presence among the major digest products of platelet enzyme of a high molecular weight fragment (apparent molecular weight 60,000). This fragment was not observed for the placental enzyme.

DISCUSSION

The suggested classification of monoamine oxidase activities into two types was based originally on their different responses to the irreversible inhibitor, clorgyline. The tyramine oxidizing activity of rat brain could be resolved into two components having pI_{50} values of 8.8 and 5.2 which were referred to as Type A and Type B activities, respectively [6].

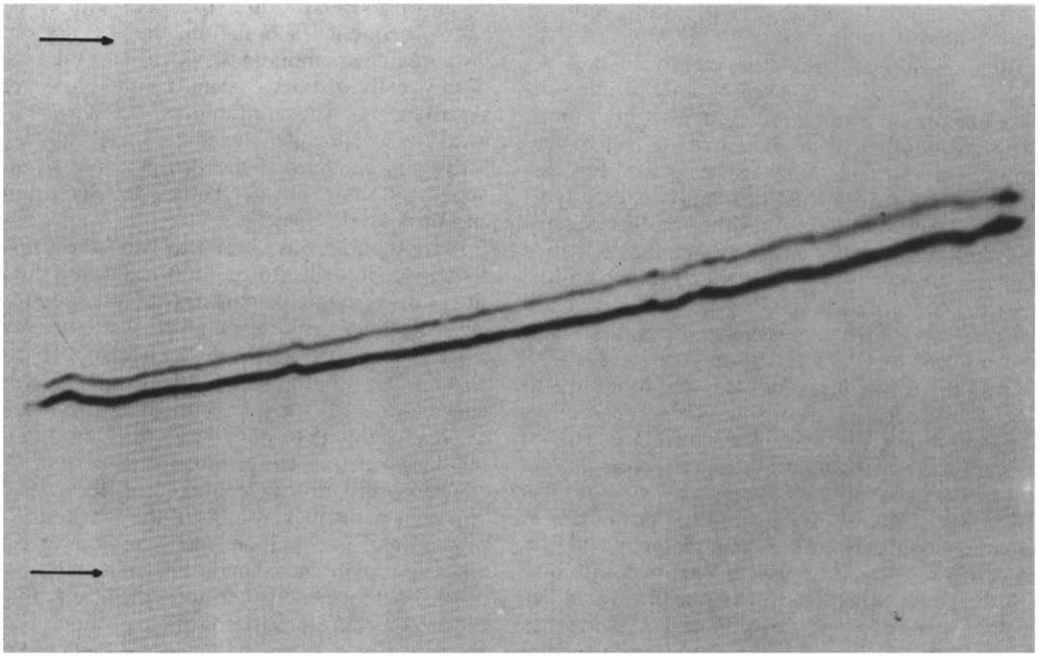

Fig. 4. Fluorograph of [^{3}H]pargyline-labelled platelet and placental monoamine oxidases resolved on a horizontal gradient polyacrylamide gel. A mixture of partially purified platelet and placental monoamine oxidase, labelled with [^{3}H]pargyline and denatured in sodium dodecylsulphate, was applied to the top of a linear horizontal gradient gel of acrylamide concentration from 5% (left) to 10% (right). Gel conditions and fluorography were otherwise as described for Fig. 3. The origin and dye front are located by arrows, the upper band is the placental enzyme and the lower one, the platelet enzyme.

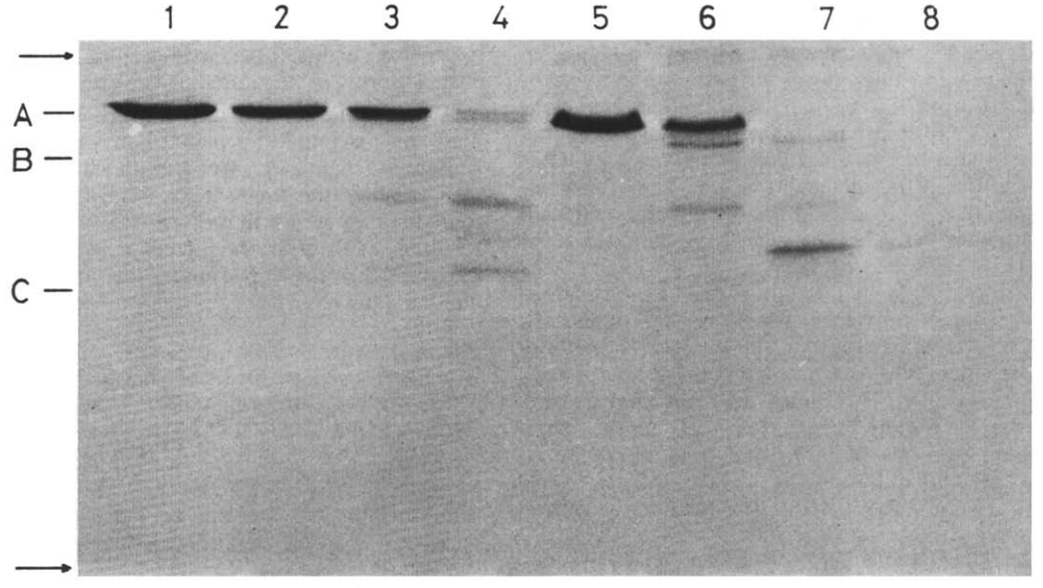

Fig. 5. Fluorograph of partial proteolytic digestion products of [^{3}H]pargyline-labelled monoamine oxidases. Preparations of [^{3}H]pargyline labelled placental and platelet monoamine oxidase were incubated for 30 min at 37° with various concentrations of protease V (*S. aureus*) in the digestion buffer of Cleveland *et al.* [24]. The partially digested fragments were then resolved by sodium dodecylsulphate–polyacrylamide gel electrophoresis (15% gel) and autoradiography as described in Materials and Methods. Results are shown for [^{3}H]pargyline-labelled placental enzyme; undigested (1) and with 0.1μg (2), 1μg (3) and 5μg (4) protease V. Corresponding results for the platelet enzyme are shown in tracks 5–8. The arrows indicate the origin and dye front and the positions A, B and C are the locations of the molecular weight markers as in Fig. 3.

These initial observations have since been considerably extended and the relationship between inhibitor sensitivity and substrate affinity noted in the original study has been more clearly defined. A-type activity can be recognised by an extreme sensitivity to clorgyline and a preferential affinity for 5-hydroxytryptamine. Type B activity is selectively inhibited by deprenil and is more active towards phenylethylamine. Both types readily oxidize tyramine and tryptamine [7, 8]. Although this classification has been useful in decribing the activities present in various tissues, the criteria on which it is based are not absolute and both types will apparently oxidize the alternative type-specific substrates, albeit at much lower rates [13, 26].

The ability of various substrates and inhibitors to distinguish two types of monoamine oxidase raises several questions concerning the interrelationship of the enzyme forms responsible for the type-characteristic behaviour. In an attempt to analyse this relationship, we have investigated the properties of monoamine oxidases from human platelets and placentae, tissues which apparently express different forms of the enzyme. There have been few studies of the biochemical properties of the enzymes in these tissues, although it is known that the placental enzyme is type A [27, 28] and the properties of the enzyme in platelets suggest that it is Type B [29]. We have characterized and compared the properties of the enzymes obtained from these two sources, both in crude membrane preparations and after similar treatments of detergent-solubilization and partial purification.

The observation that placental and platelet enzymes both retain their characteristic properties after solubilization in detergent is consistent with previous studies of monoamine oxidase solubilized and purified from bovine and rat liver [13]. However, in the present study, it was further observed that the properties of the enzymes from both sources were altered significantly under conditions of high detergent concentration. Such alterations were associated with shifts in the molecular weight of the enzyme–detergent complexes and are comparable to results obtained with the brain enzyme by Shih and Eiduson [30].

The two molecular weight forms of both platelet and placental enzymes, when isolated separately, had significantly different inhibitor sensitivities. Whereas the higher molecular weight aggregates of both enzymes had identical properties to the membrane-bound enzyme from the corresponding tissue, the lower molecular weight forms (favoured under conditions of high concentration of detergent) exhibited reduced clorgyline sensitivity and altered substrate specificity.

As the two molecular weight forms could be quantitatively interconverted by alterations in detergent concentration, it appears that they do not result from random, heterogeneous aggregation of the enzyme with other cellular components. While the presence of such components cannot be excluded, they would have to be very strongly bound to co-purify and remain stably associated during aggregation and dissociation of the enzymes.

Although the differences in the biochemical properties of the platelet and placental enzymes can be shown to persist after treatment with detergents and subsequent fractionation, they do not on their own provide unambiguous evidence for the existence of physically distinct enzyme forms. However, the persistence of such differences did suggest that it would be worthwhile to examine specifically labelled active site subunits of the two enzymes by sodium dodecylsulphate–polyacrylamide gel electrophoresis and proteolytic cleavage.

[^{3}H]Pargyline was chosen to label the active site subunits as it reacts stoichiometrically with the active site of the enzyme forming a covalently bound product [31]. Furthermore, although it is preferentially a B-type inhibitor, the difference in pI_{50} for the enzymes used in this study (10^{-6} M for the placental enzyme and 10^{-8} M for the platelet enzyme) is not as great as that determined for other, more selective inhibitors such as clorgyline.

The observation of consistent differences in the apparent molecular weights of the labelled subunits of placental and platelet monoamine oxidase provides direct evidence that the enzymes in these tissues are distinct forms. Furthermore, the observation that the electrophoretic patterns of the products obtained by proteolytic digestion of [^{3}H]pargyline-labelled enzyme are readily distinguishable, although showing some common features, suggests that the active subunits of monoamine oxidase from the two tissue sources have similar, but not identical, structures.

These results extend a recent observation published during the later stages of this investigation that the active site subunits of two forms of monoamine oxidase in rat hepatoma cells, which can be distinguished on the basis of inhibitor sensitivity, give rise to different electrophoretic profiles after proteolytic digestion [32]. However, the apparent molecular weights of the labelled subunits of the two enzyme forms in hepatoma cells were similar.

Although the physical differences described in this study on placental and platelet enzymes cannot at present be correlated directly with those properties which confer the type-specific biochemical behaviour, their existence argues against such behaviour resulting solely from the effects of local membrane environment on a single molecular form of monoamine oxidase.

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