The Amine Oxidases of Human Placenta and Pregnancy Plasma

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1. The purification of monoamine oxidase and diamine oxidase from normal human term placental tissue is described. 2. The properties of these enzymes are reported and compared with the properties of unpurified human pregnancy plasma. 3. This comparison shows that the amine oxidase of pregnancy plasma has properties corresponding to purified placental diamine oxidase, suggesting a placental origin for the plasma enzyme system. 4. Detailed kinetic study of the purified placental diamine oxidase suggests that it has a Ping Pong sequence, a mechanism of action and rate-limiting step similar to the diamine oxidase of pig kidney. 5. It is suggested that the enzyme system is important in protecting the foeto-placental unit from excesses of biogenic amines.

The presence of a monoamine oxidase (EC 1.4.3.4) (Thompson & Tickner, 1949), diamine oxidase (EC 1.4.3.6) (Danforth & Gorham, 1937) and insoluble mitochondrial monoamine oxidase (Youdim & Sandler, 1967) have been reported in human placental tissue, and the diamine oxidase has been partially purified (Smith, 1967; Paolucci *et al.*, 1971). We have isolated and purified an enzyme from normal term placentae which, though being in many ways a typical monoamine oxidase, was nevertheless able to oxidize some diamines and was found to be soluble, properties usually associated with diamine oxidase (Bardsley & Crabbe, 1973). However, the erroneous conclusion was drawn that this enzyme in fact accounted for both the diamine and monoamine oxidase activities of human placentae, but after a more detailed study of placental amine oxidases we have been able to isolate and purify an enzyme with typical diamine oxidase properties. We now report the properties of the purified monoamine oxidase and diamine oxidase of human placentae together with some observations on the amine oxidase activity of human pregnancy plasma.

Experimental

Assay of diamine oxidase activity

The direct spectrophotometric method for assaying diamine oxidase (Bardsley *et al.*, 1972) was more sensitive and versatile than the indirect methods (Holmstedt & Tham, 1959; Holmstedt *et al.*, 1961; McEwen, 1965) for the measurement of enzyme activity in plasma, during enzyme preparations and for kinetic analysis. The measurement of oxidation of substrates was by coupled oxidation of *o*-dianisidine by peroxidase and H_2O_2 as described by McEwen (1965). A number of aromatic amines

were also tested by measurement of the corresponding aldehyde produced, and where this was appreciable (*o*-, *m*- and *p*-xylylenediamines, γ -picolylamine and veratrylamine), double-reciprocal plots were obtained and K_m values calculated.

Assay of monoamine oxidase activity

The methods of Tabor *et al.* (1954) and Deitrich & Erwin (1969) were used.

Preparation of placental extract

Human placentae were obtained immediately *post partum*, the cord and membranes were removed and the placental cake was plunged into 0.9% NaCl at 4°C and manually exsanguinated. Four placentae treated in this way were homogenized at 4°C for 2 min in a Waring Blendor. The resulting brei was centrifuged at 20000*g* at 4°C for 40 min and the supernatant was used directly for the preparation of monoamine or diamine oxidase.

Preparation of placental monoamine oxidase from the placental extract

The extract from four placentae (700 ml) was made 2.55 M in $(NH_4)_2SO_4$, centrifuged at 20000*g* for 40 min and the pellet dissolved in 350 ml of 0.02 M-phosphate buffer (mixed sodium-potassium phosphate buffer, pH 7.0, was used throughout these experiments). Ethanol-chloroform (3:1, v/v) (150 ml) was added dropwise with stirring at 4°C and the resulting precipitate centrifuged at 20000*g* for 30 min. The supernatant was made 2.55 M in $(NH_4)_2SO_4$, centrifuged at 20000*g* for 40 min at 4°C and the pellet dissolved in the 0.02 M-phosphate buffer (20 ml) and applied to a column (5.5 cm $\times$ 46 cm) of CM-Sephadex

Fig. 1. Purification of human placental monoamine oxidase and diamine oxidase by column chromatography

For experimental details see the text. (a) Placental monoamine oxidase on CM-Sephadex; (b) placental monoamine oxidase on Sephadex G-200; (c) placental diamine oxidase on DEAE-Sephadex; (d) placental diamine oxidase; upward elution on Sephadex G-200. $\circ$, E_{280} , $\triangle$, E_{408} (both 10mm path length); $\bullet$, enzyme activity; $\square$, concn. of eluting buffer (M).

(CM 50). The enzyme was eluted with 0.02M buffer, the active fractions were pooled (Fig. 1a) and made 2.55M in $(\text{NH}_4)_2\text{SO}_4$. The precipitate after centrifugation was dissolved in 0.05M-phosphate buffer,

applied to a Sephadex G-200 column (2.5 cm $\times$ 130 cm) and the enzyme eluted with 0.05M-phosphate buffer (Fig. 1b). Further chromatography on CM-Sephadex resulted in a considerable increase in specific activity

(Table 1). Diamine oxidase was effectively removed by $(\text{NH}_4)_2\text{SO}_4$ precipitation after treatment with ethanol-chloroform, since, although the purified diamine oxidase is stable to $(\text{NH}_4)_2\text{SO}_4$ precipitation, it is destroyed by this procedure when carried out in the presence of traces of ethanol-chloroform mixture. The purified enzyme oxidized *p*-dimethylamino-methylbenzylamine, and since this oxidation was not affected at all by aminoguanidine even at concentrations of 1.0 mM (which completely inhibits the diamine oxidase reaction), we conclude that complete separation of the diamine oxidase from the monoamine oxidase had been achieved.

Preparation of placental diamine oxidase from the placental extract

Ethanol-chloroform (3:1, v/v) (150 ml) was added dropwise with stirring at 4°C to an extract from four placentae (700 ml). After centrifugation at 20000g for 40 min at 4°C the supernatant (650 ml) was

percolated through a column of DEAE-Sephadex (A50) (5.5 cm × 60 cm) and the column was eluted with a linear gradient of 0.02–0.4 M-phosphate buffer (Fig. 1c). The active fractions were pooled, concentrated to 10 ml by dialysis against Carbowax or sucrose, dialysed against 0.05 M-phosphate buffer and the non-diffusible material was chromatographed by upward elution on a column of Sephadex G-200 (2 cm × 70 cm) (Fig. 1d). Further chromatography on DEAE-Sephadex or hydroxyapatite resulted in a considerable increase in specific activity (Table 2) giving an enzyme that was homogeneous on polyacrylamide-gel electrophoresis. Also disc electrophoresis in the presence of sodium dodecyl sulphate (Weber & Osborn, 1969) [with β -galactosidase, bovine serum albumin, pepsin, ovalbumin and trypsin as marker proteins] gave a single band of mol.wt. 90 000, and electrophoresis as a function of pH showed the isoelectric point to be 7.1. One unit of enzyme is defined in this paper for both placental monoamine oxidase and placental diamine oxidase

Table 1. *Purification of cytoplasmic monoamine oxidase from human placenta*

For details see the text.

Step	Total volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	Yield (%)
1. Initial homogenate from four placentae	700	70000	5 (approx.)	7.14×10^{-5}	1.0	100
2. Supernatant after $(\text{NH}_4)_2\text{SO}_4$ and chloroform-ethanol precipitation	20	500	3.0	6×10^{-3}	84	60
3. Column chromatography on CM-Sephadex	2	20	1.5	7.5×10^{-2}	10.5×10^2	30
4. Column chromatography on Sephadex G-200	10	0.92	1.0	1.0	14×10^3	20
5. Further column chromatography on CM-Sephadex	10	0.5	0.8	1.6	22.4×10^3	16

Table 2. *The purification of diamine oxidase from human placenta*

For details see the text.

Step	Total volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	Yield (%)
1. Initial homogenate from four placentae	700	70000	25	2.14×10^{-4}	1.0	100
2. Supernatant after chloroform-ethanol precipitation	650	1807	15	8.3×10^{-3}	39	60
3. Column chromatography on DEAE-Sephadex	10	200	12	6.0×10^{-2}	2.8×10^2	48
4. Column chromatography on Sephadex G-200	8.5	13.3	8.0	0.6	2.8×10^3	32
5. Further chromatography on DEAE-Sephadex	5.0	6.0	6.0	1.0	4.6×10^3	24

Table 3. *Substrate specificities for placental monoamine oxidase, placental diamine oxidase and pregnancy-plasma diamine oxidase, together with values for placental diamine oxidase given by Smith (1967) and Paolucci et al. (1971)*

Assays were performed by the method of McEwen (1965), with a 3 mM substrate concentration unless otherwise indicated. Activities are expressed as a percentage of that with 1,4-diaminobutane (putrescine). — indicates that no value was obtained.

Substrate	Placental monoamine oxidase	Placental diamine oxidase	Pregnancy-plasma diamine oxidase	Placental diamine oxidase (Smith, 1967)	Placental diamine oxidase (Paolucci <i>et al.</i> , 1971)
Aliphatic monoamines					
Methylamine	82	—	62.5 (0*)	—	—
Ethylamine	91 (1 mM)	—	—	—	—
Propylamine	40	0	—	—	—
Butylamine	74	0	—	—	—
Pentylamine	20	0	61	—	—
Hexylamine	45 (1 mM)	0	—	—	—
Heptylamine	14	—	—	—	—
Octylamine	185 (1 mM)	0	100 (0*)	—	—
Nonylamine	—	0	—	—	—
Decylamine	—	0	—	—	—
Dimethylamine	40	0	—	—	—
Diethylamine	80	0	—	—	—
Trimethylamine	450	0	53.5 (44*)	—	—
Aliphatic diamines					
1,2-Diaminoethane	29	36	—	9 (2.28 mM)	7 (5 μ M)
1,3-Diaminopropane	6	14	—	42 (2.28 mM)	13 (5 μ M)
1,4-Diaminobutane	100	100	100	100 (2.28 mM)	100 (5 μ M)
1,5-Diaminopentane	84	147	31 (129*)	185 (2.28 mM)	69 (5 μ M)
1,6-Diaminohexane	42	40	—	74 (2.28 mM)	6 (6 μ M)
1,7-Diaminoheptane	—	20	—	—	6 (5 μ M)
1,8-Diamino-octane	0	30	48	—	—
1,9-Diaminononane	—	54	—	—	—
1,10-Diaminodecane	—	30	—	—	—
1,12-Diaminododecane	—	0	—	—	—
Aromatic monoamines					
Benzylamine	67	0	0	9 (2.28 mM)	0 (15 μ M)
Phenethylamine	75	0.7	33	—	0 (15 μ M)
<i>p</i> -Methylbenzylamine	—	0	—	—	—
4-Phenylbutylamine	—	0	—	—	—
Veratrylamine	—	71	0	—	—
<i>p</i> -Methoxyphenethylamine	—	0	0	—	—
<i>p</i> -Methoxybenzylamine	—	0	0	—	—
Nitrobenzylamine	—	0	0	—	—
Aromatic diamines					
<i>p</i> -Dimethylaminomethylbenzylamine	100	100	9.5 (91*)	—	—
<i>o</i> -Bis(aminomethyl)benzene	35	214	0	—	—
<i>m</i> -Bis(aminomethyl)benzene	180	50	33	—	—
<i>p</i> -Bis(aminomethyl)benzene	84	90	0 (105*)	—	—
<i>o</i> -Bis(aminoethyl)benzene	—	0	—	—	—
<i>m</i> -Bis(aminoethyl)benzene	—	0	—	—	—
<i>p</i> -Bis(aminoethyl)benzene	—	0	—	—	—
1,4-Bis(aminomethyl)cyclohexane	—	0	—	—	—
<i>o</i> -Bis-(<i>NN</i> -dimethylaminomethyl)-benzene	120	—	—	—	—
<i>p</i> -Bis-(<i>NN</i> -dimethylaminomethyl)-benzene	0	—	—	—	—
Miscellaneous					
Histamine	49	30	45	42 (2.28 mM)	85 (5 μ M)
3-Methylhistamine	270	18	37.5	—	—

Table 3 *continued*

Substrate	Placental monoamine oxidase	Placental diamine oxidase	Pregnancy-plasma diamine oxidase	Placental diamine oxidase (Smith, 1967)	Placental diamine oxidase (Paolucci <i>et al.</i> , 1971)
<i>Miscellaneous continued</i>					
1,3-Dimethyl-4(5)-(2'-aminoethyl)-imidazolium bromide	39	8.5	0	—	—
2-(Aminomethyl)pyridine	10	0	—	—	—
3-(Aminomethyl)pyridine	—	0	—	—	19 (15 μ M)
4-(Aminomethyl)pyridine	205	90	0 (110*)	—	156 (15 μ M)
Lysine	40	—	—	—	0 (15 μ M)
4-Aminobutyric acid	0	0	0	—	—
Tryptamine	14	0	0	—	0 (15 μ M)
Tyramine	185	0	24	—	0 (15 μ M)
Serotonin (5-hydroxytryptamine)	62	0	91.5 (35*)	—	0 (15 μ M)
Dopamine	650	0	510 (83*)	—	—
Adrenaline	1300	0	0	—	—
Noradrenaline	450	0	31.5	—	—
Spermine	50	32	0	—	6 (5 μ M)
Spermidine	140	0	106.5 (0*)	—	—
Mescaline	42.5	0	33	—	0 (15 μ M)
Octopamine	65	10	50	—	—

* With 2,2'-azinodi-[3-ethylbenzthiazolinesulphonic acid(6)] diammonium salt as chromogen.

as that amount of enzyme causing the oxidation of 1 μ mol of *p*-dimethylaminomethylbenzylamine/min at 37°C at a substrate concentration of 0.125 mM in air and 0.05 M-phosphate buffer, pH7.0. This corresponds to ΔE_{250} /min of 3.67.

Enzyme concentration

The enzyme concentrations used in the present study were as follows: placental monoamine oxidase 0.005 unit/ml; placental diamine oxidase, 0.02 unit/ml; pregnancy-plasma diamine oxidase, 0.00027 unit/ml.

The concentrations of both the placental monoamine oxidase and diamine oxidase approximated to those found in the placental cytoplasm under physiological conditions.

Owing to its high absorbance, it was necessary to dilute the unpurified pregnancy plasma 1:15 for spectrophotometric assays.

Kinetic isotope effect

This was studied by using *p*-dimethyl- α -bis-deuteriobenzylamine as described by Bardsley *et al.* (1973).

Results

Tables 1 and 2 show typical preparations of monoamine oxidase and diamine oxidase from human term placenta. We have found considerable variation in the activities of these enzymes in individual placenta.

(W. G. Bardsley, M. J. C. Crabbe & I. V. Scott, unpublished work), but conclude that this is not affected by the time delay in obtaining placenta, i.e. whether the placenta were obtained by section or by vaginal delivery. The most serious obstacle to purification was discovered to be blood clotting, and it was found best to avoid this by exsanguinating as soon as possible, after which the placenta could be stored indefinitely at -15°C.

Table 3 gives information on some of the most important potential substrates for these enzymes. From this information and also from results with a further 50 potential substrates it seems that placental monoamine oxidase has a typical monoamine oxidase substrate specificity, except that some diamines and polyamines were weakly oxidized. We consider that this is not due to contamination with diamine oxidase, as it is not affected by amino guanidine. It is particularly interesting that, of the aromatic amines tested (including benzylamine and *p*-dimethylaminobenzylamine), *p*-dimethylaminomethylbenzylamine was the best compound for use in a spectrophotometric assay. We had hitherto thought of this compound as more specific for diamine oxidase. The placental diamine oxidase has a substrate specificity which corresponds closely to the classical pig kidney diamine oxidase and this is also approximately true for unpurified pregnancy plasma.

The inhibitor specificities in Table 4 show that the placental monoamine oxidase and diamine oxidase correspond to classical types in this respect, except

Table 4. *Inhibition of the oxidation of p-dimethylaminomethylbenzylamine by purified human placental monoamine oxidase, diamine oxidase and unpurified pregnancy plasma*

Inhibitors	Placental monoamine oxidase (substrate concn. = 0.2 mM)		Placental diamine oxidase (substrate concn. = 0.125 mM)		Pregnancy plasma (substrate concn. = 0.04 mM)	
	Inhibitor concn.	Inhibition (%)	Inhibitor concn.	Inhibition (%)	Inhibitor concn.	Inhibition (%)
Monoamine oxidase inhibitors						
Iproniazid	0.1 mM	0	0.05 mM	66	0.05 mM	45
Harmine	0.05 mM	100	0.05 mM	80	0.1 mM	92
Phenelzine	0.1 mM	82	0.05 mM	100	—	—
Isocarboxazid	0.05 mM	0	0.05 mM	24	—	—
Isoniazid	0.05 mM	0	0.05 mM	40	0.1 mM	41.5
Nialimide	0.1 mM	93	0.05 mM	89	0.01 mM	42.5
Tranlycypamine	0.1 mM	84	0.05 mM	14	0.05 mM	23
Mebanazine	0.1 mM	93	0.05 mM	57	0.01 mM	24.5
Pargyline	—	—	—	—	0.05 mM	51.3
Carbonyl group reagents						
Aminoguanidine	1.0 mM	0	0.01 mM	100	0.05 mM	68
Hydroxylamine	0.05 mM	0	0.05 mM	100	0.1 mM	100
Semicarbazide	0.05 mM	0	0.05 mM	50	0.05 mM	37.2
Metal ion-chelating reagents						
Cuprizone	0.05 mM	>50	0.05 mM	>50	0.1 mM	30.5
Diethyldithiocarbamate	0.05 mM	100	0.05 mM	40	0.05 mM	92
EDTA	0.05 mM	67.7	0.05 mM	0	—	—
Sodium azide	0.05 mM	80.6	0.05 mM	0	0.05 mM	20
Substrate analogues						
Imidazole	0.05 mM	0	0.05 mM	57.4	0.05 mM	19
Guanidine	1.0 mM	89	0.05 mM	0	—	—
Methylguanidine	1.0 mM	98	0.05 mM	49.4	0.1 mM	22
Trimethylsulphonium iodide	1.0 mM	100	0.05 mM	0	—	—
Methylisothiuronium iodide	1.0 mM	100	0.05 mM	46.7	—	—
SH-group reagents						
Iodoacetamide	0.05 mM	100	0.05 mM	6	0.1 mM	0
N-Ethylmaleimide	0.05 mM	95.2	0.05 mM	0	0.05 mM	0
p-Chloromercuribenzoate	0.01 mM	50	0.05 mM	0	0.01 mM	0

for the unexpected inhibition of the plasma and placental diamine oxidases by so-called monoamine oxidase inhibitors.

Table 5 summarizes the properties of the monoamine and diamine oxidases and pregnancy plasma.

Fig. 1 illustrates the purification of placental monoamine and diamine oxidase and the separation from inactive protein and haemoprotein (E_{408}) achieved by column chromatography.

Fig. 2 shows the oxidation of aromatic amines by placental monoamine oxidase and diamine oxidase as determined spectrophotometrically. Most of the amines studied would be of limited value in spectrophotometric assay procedures owing to the high degree of substrate inhibition given by them. From these double-reciprocal plots it seems that *p*-dimethylaminomethylbenzylamine is the most useful substrate for spectrophotometric assay of both placental monoamine and diamine oxidase and pregnancy plasma. A development of this method (W. G.

Bardsley, M. J. C. Crabbe & I. V. Scott, unpublished work) for assay of pregnancy-plasma amine oxidase has been successfully accomplished and is now in use for routine screening of pregnant women.

Fig. 3 shows that double-reciprocal plots with several values for the fixed substrate are a family of parallel lines whichever substrate is fixed. This suggests a catalytic sequence involving only binary enzyme-substrate complexes, i.e. a Ping Pong Bi Ter mechanism.

The product-inhibition patterns shown in Fig. 4 (aminoaldehyde, non-competitive; H_2O_2 , uncompetitive; NH_3 , competitive) are in accordance with an obligatory ordered release of products where the aminoaldehyde is released first (P) followed by H_2O_2 (Q) and NH_3 (R) (see the Discussion section).

To assess the possible effect on the placenta of medication with monoamine oxidase inhibitors and thus provide information which may be of value in management of patients, it was decided to assess the

Table 5. Comparison of the properties of purified human placental monoamine oxidase, diamine oxidase and pregnancy plasma

	Monoamine oxidase 6.9	Diamine oxidase 7.45	Pregnancy plasma 7.6
pH optimum			
K_m values for:			
<i>o</i> -Xylylene diamine	—	0.215 mM	—
<i>m</i> -Xylylene diamine	0.28 mM	0.714 mM	0.029 mM
<i>p</i> -Xylylene diamine	0.045 mM	0.027 mM	0.25 mM
<i>p</i> -Dimethylaminomethylbenzylamine	0.143 mM	0.1 mM	0.06 mM
<i>p</i> -Dimethyl- $\alpha\alpha$ -bisdeuteriobenzylamine	0.37 mM	0.166 mM	0.121 mM
4-Aminomethylpyridine	0.0394 mM	—	0.0481 mM
Veratrylamine	—	5.5 mM	—
Histamine	1.25 mM	—	—
Thermal stability	Rapidly destroyed at 50°C	Stable at 50°C	Stable at 50°C
Freezing and thawing	—	Stable	Stable
Ethanol-chloroform treatment ($(\text{NH}_4)_2\text{SO}_4$)	Stable Stable before and after ethanol-chloroform treatment	Stable Stable only in the absence of ethanol-chloroform	Stable Stable only in the absence of ethanol-chloroform
Chromatographic behaviour	Held weakly on DEAE-Sephadex	Held strongly on DEAE-Sephadex	Held strongly on DEAE-Sephadex
Effect of added co-factor			
Addition of FMN, FAD	No effect	No effect	No effect
Addition of pyridoxal phosphate	Rate enhancement	Rate enhancement	Rate enhancement
Deuterium isotope effect (with air as the gaseous phase)	$k_H/k_D = 6.3$ (0.1 mM)	$k_H/k_D = 2.1$ (0.1 mM)	$k_H/k_D = 1.4$ (0.1 mM)
	$V_{\text{max.H}}/V_{\text{max.D}} = 4.0$	$V_{\text{max.H}}/V_{\text{max.D}} = 1.63$	$V_{\text{max.H}}/V_{\text{max.D}} = 1.23$
Substrates	Monoamines, particularly catecholamines. Histamine and diamines weakly oxidized	Histamine and diamines. Not monoamines	Monoamines and diamines
Inhibitors	Monoamine oxidase inhibitors, -onium compounds, SH-group reagents and copper ion-chelating agents	Carbonyl group reagents, copper chelating agents and monoamine oxidase inhibitors.	Strongly inhibited by carbonyl-group reagents and weakly by monoamine oxidase inhibitors

quantitative effect of monoamine oxidase inhibitors on the purified placental monoamine oxidase. This information is displayed in Fig. 5. The inhibition produced by tranlycypamine is strictly competitive, but not a linear function of inhibitor concentration and so at low substrate concentration considerable inhibition is produced at inhibitor concentrations greater than 0.02 mM (e.g. 50% inhibition is given by 0.02 mM inhibitor at a substrate concentration of 0.23 mM, by 0.05 mM inhibitor at 0.58 mM substrate and 0.08 mM inhibitor at 1.42 mM substrate concentration).

Further studies (M. J. C. Crabbe & W. G. Bardsley, unpublished work) indicate the surprising result that many of the better known monoamine oxidase inhibitors also inhibit the diamine oxidases from both the human placenta and pig kidney.

Fig. 6 indicates the inhibition of placental diamine oxidase by 1,10-phenanthroline. According to a

study of the effect of metal ion-chelating agents on metalloenzymes (Bardsley & Childs, 1974), if the chelating agent removes y metal ions from the enzyme to form a chelate complex where the ratio of chelating agent to metal ion is x , then with the degree of dissociation of metalloenzyme = λ we have:

$$\lambda \left(\frac{\lambda}{1-\lambda} \right)^{\frac{1}{y}} = \frac{K_1^{\frac{1}{y}}}{y[E_0]} (1 + K_2[I]^x)$$

In this equation $[E_0]$ is the enzyme concentration, K_1 the equilibrium constant for dissociation of metal ions from the enzyme according to the equation:

where E_0 is metalloenzyme, E represents enzyme without metal ions and M represents metal ions, and K_2 is the equilibrium constant for the reaction:

Fig. 2. Double-reciprocal plots for aromatic amines obtained by spectrophotometric determination of the corresponding aldehydes

For experimental details see the text. (a) Placental monoamine oxidase; (b) placental diamine oxidase; (c) unpurified pregnancy plasma. ○, *p*-Dimethylaminomethylbenzylamine; △, *p*-dimethylaminomethyl- $\alpha\alpha$ -bisdeuteriobenzylamine; □, *m*-bis(aminomethyl)benzene; ●, *p*-bis(aminomethyl)benzene; ▲, 4-aminomethylpyridine; ■, veratrylamine.

Fig. 3. Effect of varying the concentration of both substrates on initial rates with placental diamine oxidase

For experimental details see the text. (a) p -Dimethylaminomethylbenzylamine as the varied substrate (A) against fixed O_2 concentrations. $\circ$, 0.97 mm; Δ , 0.291 mm; $\square$, 0.138 mm; $\bullet$, 0.097 mm; $\blacktriangle$, 0.0415 mm; $\blacksquare$, 0.0138 mm. (b) O_2 as the varied substrate (B) against fixed concentrations of p -dimethylaminomethylbenzylamine. $\circ$, 4 mm; Δ , 1.25 mm; $\square$, 0.4 mm; $\bullet$, 0.2 mm; $\blacktriangle$, 0.125 mm. (c) Intercepts from Figs. 3(a) and 3(b) replotted for $\circ$, amine fixed and O_2 varied, and Δ , O_2 fixed and amine varied. This gives $K_a = 1.4$ mm and $K_b = 0.29$ mm for the initial rate v given by:

$$v = \frac{V [A] [B]}{K_a [B] + K_b [A] + [A] [B]}$$

when no products are present.

where I is the chelating agent and $[MI_x]$ the chelate complex. According to this theory, the degree of dissociation of the metalloenzyme $\lambda = [1 - ([E]/[E_0])]$ can be determined by assuming that enzyme activity in the absence of inhibitor is proportional to $[E_0]$ and in the presence of inhibitor is proportional to $[E]$. Our results with the diamine oxidase of pig kidney (Bardsley *et al.*, 1974) show that this enzyme reacts instantaneously with chelating agent to reach an equilibrium position dictated solely by inhibitor concentration. A plot of λ against $[I]$ was sigmoid, and it

was found that $2Cu^{2+}$ ions were removed to form chelate complexes which in all cases had the maximum ratio of chelating agent to metal ion, i.e. K_2 was the cumulative equilibrium constant for the chelating system.

The results with 1,10-phenanthroline show that placental diamine oxidase behaves in exactly the same way as the pig kidney diamine oxidase. The least-squares regression coefficient for $x = 3$ and $\lambda[\lambda/(1-\lambda)^{1/y}]$ was for $y = 2$, 0.203 and for $y = 3$, 0.1505. With $[E_0] = 1.0 \text{ nm}$ and assuming the metal to be

Fig. 4. Use of products as inhibitors of the oxidation of *p*-dimethylaminomethylbenzylamine by placental diamine oxidase with air as the gaseous phase

For experimental details see the text. (a) *p*-Dimethylaminomethylbenzaldehyde as product inhibitor; $\circ$, no inhibitor; Δ , 0.1 mm inhibitor; $\square$, 0.2 mm inhibitor; $\bullet$, 0.3 mm inhibitor; $\blacktriangle$, 0.35 mm inhibitor; $\blacksquare$, 0.4 mm inhibitor. (b) Non-competitive inhibition by *p*-dimethylaminomethylbenzaldehyde illustrated by a replot of slopes and intercepts as a function of inhibitor concentration. $\circ$, Slopes; Δ , intercepts. (c) H_2O_2 as product inhibitor; $\circ$, no inhibitor; Δ , 0.5 mm inhibitor; $\square$, 0.75 mm inhibitor; $\bullet$, 1.0 mm inhibitor; $\blacktriangle$, 1.5 mm inhibitor; $\blacksquare$, 2.0 mm inhibitor. (d) Uncompetitive inhibition by H_2O_2 illustrated by replot of intercept ($\circ$) as a function of inhibitor concentration. (e) NH_3 as product inhibitor; $\circ$, no inhibitor; Δ , 5 mm inhibitor; $\blacksquare$, 15 mm inhibitor; $\bullet$, 30 mm inhibitor; $\blacktriangle$, 50 mm inhibitor; $\blacksquare$, 70 mm inhibitor. (f) Competitive inhibition by NH_3 illustrated by a replot of slope ($\circ$) as a function of inhibitor concentration. This gives $K_i = 9.0 \text{ mm}$ for NH_3 .

Fig. 5. Inhibition of placental monoamine oxidase by tranlycypamine

(a) Double-reciprocal plots illustrating the oxidation by placental monoamine oxidase of *p*-dimethylaminomethylbenzylamine in air ($\circ$) and with final concentrations of tranlycypamine of Δ , 0.02 mm ; $\square$, 0.05 mm ; $\bullet$, 0.08 mm . (b) Competitive inhibition of placental monoamine oxidase by tranlycypamine illustrated by replottting the slope effect as a function of inhibitor concentration.

Fig. 6. Effect of 1,10-phenanthroline on placental diamine oxidase

(a) The percentage activity of the uninhibited reaction as a function of time after incubation with 1,10-phenanthroline at a final concentration of: $\circ$, 0.22 mM; Δ , 0.15 mM; $\square$, 0.125 mM; $\bullet$, 0.075 mM. After incubation with 1,10-phenanthroline at 20°C for the time indicated, *p*-dimethylaminomethylbenzylamine was added to give a final concentration of 0.125 mM. Initial rates were linear and carrying out experiments under various conditions indicated that the reaction rate was dictated solely by the final concentration of chelating agents and substrate. (b) The degree of dissociation (λ) of metal ions from the enzyme [presumed to be given by $1 - ([E]/[E_0])$], where $[E]$ is proportional to enzyme activity in the presence of chelating agent and $[E_0]$ is proportional to enzyme activity in the absence of chelating agent] as a function of final chelating-agent concentration. (c) A plot of the function $\lambda \{ \lambda / (1 - \lambda) \}^{1/2}$ for $\circ$, $y = 2$ and Δ , $y = 3$ against final concentration of chelating agent. The solid line is a least-squares cubic fitted to the points for $y = 3$ and the dashed line is a least-squares cubic fitted to the experimental points for $y = 2$.

Cu^{2+} , K_2 for 1,10-phenanthroline would be 10^{21} and our results would indicate either 2Cu^{2+} ions with $K_1 = 4.06 \times 10^{-36} \text{ mol} \cdot \text{litre}^{-1}$ or 3Cu^{2+} ions with $K_1 = 9.2 \times 10^{-56} \text{ mol} \cdot \text{litre}^{-1}$.

Discussion

It seems clear from the results of the present paper that the human placenta elaborates two soluble amine oxidases. One is a diamine oxidase which

appears to resemble the classical diamine oxidase of pig kidney in its properties, and the other is an enzyme with typical monoamine oxidase properties except that it is soluble, cytoplasmic in origin and oxidizes many diamines, albeit rather weakly compared with the best substrates (catecholamines). The properties of the diamine oxidase of pregnancy plasma seem closely similar to those of placental diamine oxidase, and this supports the hypothesis that the plasma

enzyme activity does in fact originate in the placenta. We have some reservations about this conclusion, however, since diamine oxidase activities in plasma may not correlate with activities in individual placentae and, more seriously, diamine oxidase activity can be detected in some non-pregnant women and sometimes even in males (W. G. Bardsley, M. J. C. Crabbe & I. V. Scott, unpublished work).

It is of some interest that the kinetic study of the purified placental diamine oxidase seems to indicate a Ping Pong Bi Ter mechanism with the sequence:

where A is diamine, B is oxygen, P is aminoaldehyde, Q is H_2O_2 and R is NH_3 . Further, the deuterium isotope effect locates the rate-determining step in the sequence $EA \rightarrow FP$, and this indicates that the enzyme is closely similar to the diamine oxidase of pig kidney (Bardsley *et al.*, 1973). It is of interest that the kinetic isotope effect with the placental diamine oxidase was much smaller than that with the placental monoamine oxidase, which may have some mechanistic significance. An interesting feature of the product-inhibition patterns is that the slope and intercept replots for the aminoaldehyde and H_2O_2 indicate that the inhibition is described by a polynomial in inhibitor concentration of degree greater than one, i.e. the product inhibition is also accompanied by dead-end inhibition and/or partial inhibition.

We have discussed the possible functional significance of this enzyme system in pregnancy (Bardsley & Crabbe, 1973) as a protection for the foeto-placental unit. Concentrations of biogenic amines could become elevated if the oxygen partial pressure in the placenta fell too low, leading to placental damage, and it is important to inquire as to the utilization of O_2 by these enzymes. Some information is now available about the placental diamine oxidase. This has an apparent K_m for O_2 of 0.29 mM. Defining V_{max} as the velocity with saturating conditions of both O_2 and amine, then with amine saturating the percentage of V_{max} given at various O_2 concentrations would be approximately air (50% V_{max}), 3% O_2 (18% V_{max}) and 1% O_2 (8% V_{max}). The normal range of O_2 partial pressure encountered in

pregnancy is 11.8% O_2 ($pO_2 = 12000$ Pa) in the maternal arterioles to 2.6% O_2 ($pO_2 = 2700$ Pa) in the umbilical artery. It is thus obvious that diamine oxidase is operating in the placenta within a fairly critical oxygen concentration range. Any fall in O_2 partial pressure owing to diminished circulation could render the protective function ineffective, leading to polyamine accumulation and subsequent compromise to the vasomotor stability of the placental microcirculation.

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