Biochemical Pharmacology, Vol. 21, pp. 2197–2204. Pergamon Press, 1972. Printed in Great Britain.

BEEF ADRENAL MEDULLA MONOAMINE OXIDASE

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(*Received* 19 *January* 1972; *accepted* 7 *March* 1972)

Abstract—Beef adrenal medulla monoamine oxidase has been solubilized and purified 24-fold and its properties have been compared with those of a preparation of the rat liver enzyme which had been solubilized in a similar way. The two enzyme preparations exhibited similar substrate specificities, molecular weights and pH optima. The "isoenzymes" which could be separated by polyacrylamide gel electrophoresis were found to be similar in distribution in these two enzyme preparations. The individually separated isoenzyme bands were found to contain considerably different amounts of phospholipid. The species that moved towards the cathode on polyacrylamide gel electrophoresis was found to have a relatively high specific activity with dopamine as the substrate and to differ from the remainder of the enzyme in being relatively insensitive to inhibition by the hydrazine inhibitors 2-phenylethylhydrazine, iproniazid and isocarboxazide.

THE ENZYME monoamine oxidase (Monoamine:O_2 oxidoreductase (deaminating) EC 1.4.3.4) has been shown to be present in both the adrenal cortex[1,2] and medulla.[3,4] In the medulla the enzyme has been shown to be mainly mitochondrial in localization with little activity being associated with the chromaffin granules.[5–7] The enzyme from beef adrenal medulla has been shown to be active against tyramine[5,6] and tryptamine[7] but no information concerning the specificity of the enzyme has been reported.

Monoamine oxidase has been prepared from a number of different tissues and considerable differences between these preparations have been reported.[8] Monoamine oxidase requires vigorous treatment in order to extract it from the mitochondrion and no detailed comparison of the effects of the extraction procedure used on the properties of the enzyme has been made. In this paper the properties of monoamine oxidase from beef adrenal medulla are compared with those of the enzyme from rat liver in preparations which have both been solubilized in the same way.

MATERIALS AND METHODS

Purification of beef adrenal medulla monoamine oxidase

Beef adrenal glands were collected immediately after slaughter and were kept in ice for transport to the laboratory. The cortex was separated from each gland and was discarded. The medullas were suspended in approximately 10 vol. (wt./vol.) of ice-cold 0·25 M sucrose containing 2·5 mM tris, HCl buffer (pH 7·4) and the mixture was homogenized using a Dounce homogenizer. The homogenate was centrifuged at 1500 *g* for 10 min at 4° and the supernatant was carefully decanted. This supernatant was centrifuged at 10,000 *g* for 10 min at 4° and the sediment was resuspended in half

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the original volume of the sucrose medium. The suspension was resedimented by centrifugation at 10,000 *g* for 10 min at 4° and the sediment, which was the crude mitochondrial fraction, was suspended in about one-quarter of the original volume of 0·01 M sodium phosphate buffer, pH 7·2 and was stored frozen.

Monoamine oxidase was extracted from the mitochondrial fraction by the effects of sonic oscillations in the presence of benzylamine followed by treatment with 1·25% (wt./vol.) of the nonionic detergent Triton X-100 and centrifugation. This procedure was identical to that described for the solubilization of rat liver mitochondrial monoamine oxidase.[9] The solubilized enzyme was then cooled in ice and fractionated with ammonium sulphate. The material precipitating between 30 and 65 per cent saturation, was removed by centrifugation and dissolved in a minimum volume of 0·05 M sodium phosphate buffer, pH 7·4. This solution was then passed through a column of Sephadex G-25 (coarse) which had been equilibrated with the same buffer.

The active material from the Sephadex column was then applied to a 7 × 2 cm column of DEAE–cellulose which had been equilibrated with the 0·05 M sodium phosphate buffer pH 7·4. The column was washed at room temperature with five column volumes of the equilibration buffer and then with five column volumes of the same buffer containing 0·1 M sodium chloride. The active enzyme was then eluted from the column with the same buffer containing 0·2 M sodium chloride.

The enzyme prepared in this way could be concentrated by precipitation with ammonium sulphate (to 65 per cent saturation) followed by resuspension in the appropriate buffer. The enzyme preparation was stable for several weeks at 4°. A summary of the purification procedure, which resulted in about a 25-fold purification is shown in Table 1.

TABLE 1. PURIFICATION OF BEEF ADRENAL MEDULLA MONOAMINE OXIDASE

Purification Step	Volume (ml)	Total protein (mg)	Specific activity (units/mg of protein)	Total activity (Units)	Yield (%)	Purification
Mitochondria	100	3680	0·0117	43	100	
Solubilized enzyme	102	1480	0·033	49	114	2·9
Ammonium Sulphate (30–65% saturation)	15	174	0·082	14·3	33·4	7·0
DEAE–Cellulose eluate	15·8	22·6	0·28	6·3	14·7	23·9

Assay methods. Enzyme activity was assayed during purification using the spectrophotometric method of Tabor, Tabor and Rosenthal[10] which employs benzylamine as the substrate. Determinations of kinetic parameters towards different substrates were made using an oxygen electrode as previously reported.[11] The oxygen electrode was also used to determine the activities of bands of enzyme activity eluted from polyacrylamide gels except when dopamine was used as the substrate, in which case the radiochemical method of Robinson *et al.*[12] as adapted by Southgate and Collins[13] was used. All assays were conducted at 30° and specific activity is defined as the number of μmoles of product formed or μatoms of oxygen consumed/min/mg of enzyme protein.

Electrophoresis. Polyacrylamide gel electrophoresis was carried out at pH 9·1 in the manner described by Youdim *et al.*[14] and monoamine oxidase activity was located

in the gels, with tryptamine as the substrate, using the method of Glenner *et al.*[15] The separated bands of enzyme activity were eluted from unstained gels by extraction of 4° with 0·01 M sodium phosphate buffer pH 7·2.

Other methods. Rat liver monoamine oxidase was partly purified by a simplified procedure taken from the more complete purification described by Youdim and Sourkes[9] as previously reported.[16] Protein concentration was estimated by the microbiuret method[17] using bovine serum albumin as a standard. Phospholipid was extracted by the method of Folch *et al.*[18] and phosphorus was determined by the method of Michelsen.[19]

The approximate molecular weight of the enzyme was determined by gel-filtration of a sample on a 35 × 2·5 cm column of Sephadex G-200, equilibrated with 0·01 M sodium phosphate buffer, pH 7·2, using the method of Determann and Michel.[20]

Chemicals. 2-[^{14}C]Dopamine was obtained from the Radiochemical Centre, Amersham. Whatman DEAE–cellulose (DE-52) was obtained from H. Reeve Angel Ltd. and Sephadex G-25 (coarse) and G-200 were obtained from Pharmacia (London) Ltd. Iproniazid (Marsilid) and isocarboxazide (Marplan) were kindly given by Roche Products Ltd., Welwyn Garden City, 2-phenylethylhydrazine was obtained from Fluka A.G., Switzerland and tranylcypromine was a kind gift from Lakeside Laboratories Inc., Milwaukee, U.S.A. Dopamine and the *m*-O-methylated derivatives of adrenaline and noradrenaline were obtained from Koch–Light Laboratories Ltd., Colnbrook. All other chemicals were obtained from B.D.H. Ltd., Poole. Distilled water was passed through a Permutit Mark 11 deionizer before use.

RESULTS

The approximate molecular weight of the enzyme was estimated to be 300,000 by gel-filtration on Sephadex G-200. No significant difference in the elution volumes of enzyme activity could be detected between the beef adrenal medulla and the rat liver monoamine oxidase preparations.

The variation of the activity of the beef adrenal medulla enzyme with pH is shown in Fig. 1 with benzylamine as the substrate. The pH optimum is at about pH 8·5.

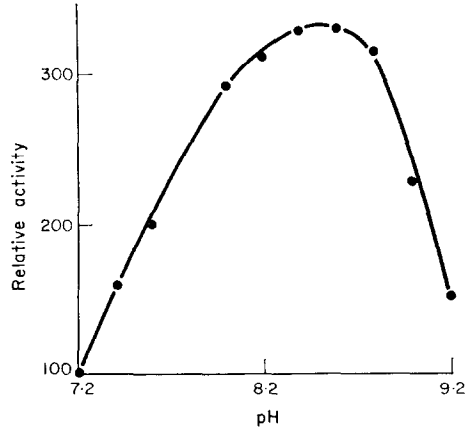

FIG. 1. The effect of pH on the activity of beef adrenal medulla monoamine oxidase. Activity towards benzylamine was determined spectrophotometrically by the method of Tabor *et al.*[10] All assays were carried out in 0·1 M Tris–HCl buffer. Activities are expressed relative to that at pH 7·2 which is taken to be 100.

Specificity. Values for the Michaelis constants for the oxidation of a number of substrates and values for the maximum velocities, relative to that for tyramine, are compared with those previously reported for the rat liver enzyme[21] in Table 2.

TABLE 2. SUBSTRATE SPECIFICITIES OF BEEF ADRENAL MEDULLA AND RAT LIVER MONOAMINE OXIDASES

Substrate	Beef adrenal medulla		Rat liver	
	$K_m(\mu M)$	V_{max}	$K_m(\mu M)$	V_{max}
Oxygen	285		312	
Tyramine	840	100	870	100
Tryptamine	1050	44	980	47
Serotonin	65	15		
Adrenaline	1400	16	1390	13
Noradrenaline	350	21	392	20
m-O-Methyl adrenaline	140	16	120	15
m-O-Methyl noradrenaline	51	18		

The K_m values for the amines were determined at 30° in 0·2 M phosphate buffer pH 7·2 at an oxygen concentration of 0·23 mM. The K_m values for oxygen were determined with 5 mM tyramine as the second substrate. V_{max} values are expressed relative to that of tyramine which is taken as 100.

Electrophoresis. A comparison of polyacrylamide gel electrophoretograms of beef adrenal medulla and rat liver monoamine oxidases after staining for activity is shown in Fig. 2. In both cases a single band of activity migrates toward the cathode (designated the cathodic band) whilst one band remains at the origin and three bands migrate toward the anode. These latter four bands are here designated the anodic bands 1–4, numbering from the origin as suggested by Youdim *et al.*[14]

The specific activities of the individual electrophoretically separated bands towards tyramine, tryptamine and dopamine are shown in Table 3. The intensity of staining of the individual bands of monoamine oxidase activity when the histochemica

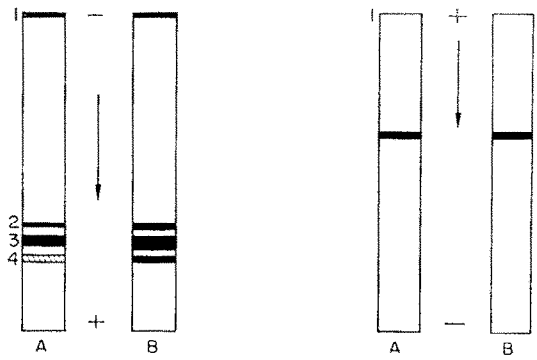

FIG. 2. Polyacrylamide gel electrophoresis of monoamine oxidase. 200 μl of the enzyme preparation was subjected to electrophoresis on a 5% polyacrylamide gel using a continuous 0·05 M Tris–HCl buffer system of pH 9·1. The initial current was 6 mA per tube and fell during the run. Enzyme activity was detected in the gel using the histochemical stain of Glenner *et al.*[13] Results for both the beef adrenal medulla (A) and rat liver (B) enzymes are shown.

stain[12,13] is used does not reflect the activities of these components when they are extracted from the gel. Thus band 4 shows considerably weaker staining than band 2 with tryptamine as the substrate, although the activities of the extracted bands show no great difference with this substrate (Table 3).

TABLE 3. THE ACTIVITIES OF ELECTROPHORETICALLY SEPARATED FORMS OF BEEF ADRENAL MEDULLA MONOAMINE OXIDASE

	Specific activity		
Component	Tyramine	Tryptamine	Dopamine
Cathodic	0·018	0·020	0·116
Anodic 1	0·35	0·22	<0·01
Anodic 2	0·24	0·14	<0·01
Anodic 3	0·09	0·06	<0·01
Anodic 4	0·14	0·18	<0·01

Specific activities are expressed as n-atoms of oxygen consumed or n-moles of product formed/mg/min. The anodic bands are numbered from the origin.[14]

The phospholipid contents of each of the separated anodic bands are shown in Table 4. Unfortunately it was not found possible to obtain sufficient of the cathodic material to allow accurate determination of the phospholipid phosphate content to be made.

TABLE 4. PHOSPHOLIPID CONTENT OF THE ELECTROPHORETICALLY SEPARATED FORMS OF BEEF ADRENAL MEDULLA MONOAMINE OXIDASE

	Anodic			
Component	1	2	3	4
Phospholipid phosphorus (μg/mg protein)	0·32	0·17	0·10	0·05

The components are numbered from the origin.[14]

Effects of inhibitors. The time courses of inhibition of the activity of beef adrenal medulla monoamine oxidase by 2-phenylethylhydrazine when a number of different substrates were used are shown in Fig. 3. The activity towards dopamine appears to be relatively insensitive to this inhibitor under the conditions used whereas there was no detectable difference between the sensitivities of the activities towards the other substrates used. Qualitatively similar results were obtained when the monoamine oxidase inhibitors iproniazid and isocarboxazide were used. In contrast when tranylcypromine was used as the inhibitor no significant difference could be seen between the time courses of inhibition of activity towards tyramine and dopamine.

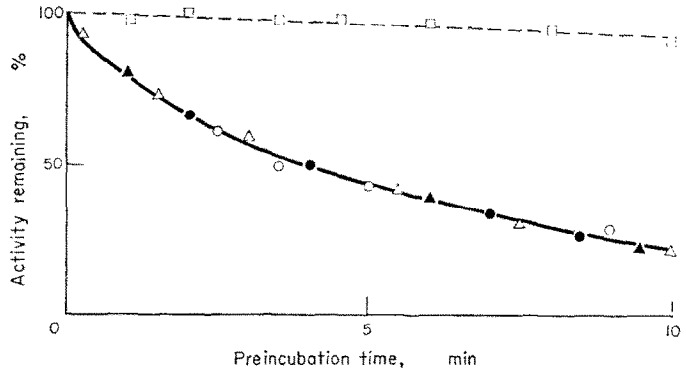

FIG. 3. The inhibition of beef adrenal medulla monoamine oxidase by 2-phenylethylhydrazine. The enzyme was preincubated at 30°, in 0·2 M phosphate buffer, pH 7·2, with 33 μM 2-phenylethylhydrazine for the indicated time before the activity was determined. The substrates used were kynuramine (●), tyramine (○), serotonin (△), benzylamine (▲) and dopamine (□).

DISCUSSION

The partly purified preparation of beef adrenal medulla monoamine oxidase appears to be similar in many of its properties to the enzyme purified from rat liver by Youdim and Sourkes.[9] The approximate molecular weight determined by gel filtration of the beef adrenal medulla enzyme is similar to that reported for the rat liver preparation[9] and in this study no significant difference between the elution volumes of these two preparations, from a column of Sephadex G-200, could be detected. The pH optimum for the beef adrenal medulla enzyme is close to that reported for the rat liver enzyme[22] and there appears to be little difference between the substrate specificities and isoenzyme distributions of these two preparations. These close similarities are somewhat surprising since there appear to be considerable species and organ differences between monoamine oxidase preparations from other sources.[8]

The K_m value for oxygen of the beef adrenal medulla enzyme is similar to those previously reported for monoamine oxidase preparations from pig brain,[23] beef liver,[24] rat liver[21] and beef thyroid.[25] The K_m value determined is of the same order as the oxygen content of air saturated water, and the possible implications of this in terms of the *in vivo* function of monoamine oxidase have recently been discussed.[21]

The presence of multiple forms of monoamine oxidase that can be separated by electrophoresis has been reported for preparations from several sources including rat liver,[14,26] although preparations from other sources have been found to apparently contain only a single enzyme species.[27–30] The phospholipid contents of the separated bands of the beef adrenal medulla monoamine oxidase are very different (Table 1). The contents of the separated bands are similar to those previously reported by Tipton for the multiple forms of the enzyme from rat liver.[21] It is, however, evident from the gradation of phospholipid phosphate content between the bands, that the presence of varying amounts of phospholipid phosphate groups cannot account for the differing electrophoretic mobilities of the components simply in terms of charge.

Of the electrophoretically separated components, the cathodic band appears to contain the majority of the activity towards dopamine, a finding which is in agreement with results previously reported for preparations from human brain,[26,31] rat brain[26]

and rat liver.[21,26] In common with results previously reported for the human brain and rat liver preparations the cathodic component from the beef adrenal medulla preparation is relatively insensitive to inhibition by the substituted hydrazine class of monoamine oxidase inhibitors, such as iproniazid, isocarboxazide and 2-phenylethylhydrazine. In contrast, no detectable differences between the sensitivities of the oxidase activity towards a number of other substrates could be detected when the beef adrenal medulla enzyme was incubated with 2-phenylethylhydrazine, a result which is similar to that previously reported for the rat liver enzyme.[30] In contrast the non-hydrazine monoamine oxidase inhibitor tranylcypromine was found to cause appreciable inhibition of the activity towards dopamine as had previously been found with preparations from rat liver[21,26] and human brain.[31]

The cathodic component of monoamine oxidase preparations from three different sources therefore appears to differ in having a relatively high specificity for dopamine and a considerably different inhibitor specificity from the remainder of the enzyme.

The exact nature of the multiple forms of monoamine oxidase preparations that can be separated electrophoretically is unclear, although the different phospholipid contents may add some credence to the suggestion by Veryovkina *et al.*[32] that the multiple forms could be due to varying amounts of membrane material bound to a single enzyme (cf. Ref. 21 for discussion of this theory). The observation that no separable bands of activity can be obtained when rat liver monoamine oxidase is subjected to polyacrylamide gel electrophoresis in Triton[21] would be also in accord with this interpretation. The possibility that the isoenzymes may be due to the presence of varying amounts or types of membrane material bound to a single enzyme species does not necessarily imply that multiple forms of the enzyme do not exist *in vivo*. Variations in the extents of inhibition of the different forms have been shown to result from pretreatment with drugs *in vivo*[26] and neuronal and extra-neuronal monoamine oxidases have been reported to have different substrate specificities.[33,34] It has been shown that, after extraction with butan-2-one, pig liver monamine oxidase has an extremely high affinity for cardiolipin[35,36] and it may be that in the cell the amount and type of lipid bound may depend on the environment of the enzyme. In this connexion the report that mitochondria from rat brain are heterogeneous and may be separated into four species each having monoamine oxidase activity is particularly interesting.[37] A final decision on the nature of the multiple forms of monoamine oxidase must, however, await a more detailed characterization of the separated forms of the enzyme.

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