Purification and Immunochemical Characterization of Monoamine Oxidase from Rat and Human Liver

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1. Monoamine oxidase from rat and human liver was purified to homogeneity by the criterion of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. 2. The enzyme activity was extracted from mitochondrial preparations by Triton X-100. The enzyme was purified by $(\text{NH}_4)_2\text{SO}_4$ fractionation followed by chromatography on DEAE-cellulose, Sepharose 6B, spheroidal hydroxyapatite, and finally chromatography on diazo-coupled tyramine-Sepharose. 3. Distinct differences occur in the chromatographic behaviour of the two enzymes on both DEAE-cellulose and spheroidal hydroxyapatite. 4. It is unlikely that the purification of the enzymes on tyramine-Sepharose is due to affinity chromatography and reasons for this are discussed. 5. The purified enzymes did not oxidize 5-hydroxytryptamine and the relative activities of the enzymes with benzylamine were increased approx. 1.25-fold compared with the enzyme activities of mitochondrial preparations. 6. Immunotitration of enzyme activity in extracts of mitochondrial preparations from rat liver was carried out with 5-hydroxytryptamine, tyramine and benzylamine. The enzyme activities were completely immunoprecipitated by the same volume of antiserum. Similar results were obtained with the antiserum to the enzyme from human liver.

Monoamine oxidase [monoamine- O_2 reductase (deaminating), EC 1.4.3.4] has been isolated and partially purified from many tissues of various species (see Sandler & Youdim, 1972; Gorkin, 1973 for reviews). The work reported here describes the purification of monoamine oxidase from rat and human liver and subsequent immunochemical studies on the enzymes.

Monoamine oxidase has been purified from ox liver (Nara *et al.*, 1966; Gomes *et al.*, 1969) and human liver (Norstrand & Glantz, 1973) but the methods used are extremely complicated and time-consuming. The procedure described here involves modification of the existing purification schemes (Nara *et al.*, 1966; Gomes *et al.*, 1969; Youdim & Collins, 1971; Houslay & Tipton, 1973; McCauley & Racker, 1973; Houslay & Tipton, 1975) with the addition of a final chromatographic step on diazo-coupled tyramine-Sepharose. Monoamine oxidase can be purified considerably from Triton X-100 extracts of mitochondrial preparations by chromatography on tyramine-Sepharose (Dennick & Mayer, 1976). When used in combination with conventional purification procedures the method gives preparations of monoamine oxidase from rat and human liver that are homogeneous by the criterion of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate.

Antisera to the purified enzymes have been used to assess the relationship of the two forms of monoamine oxidase which are functionally defined by substrate specificity.

Materials and Methods

Animals

Adult Wistar rats and sheep were obtained from the Joint Animal Breeding Unit, University of Nottingham School of Agriculture, Sutton Bonington, Leics., U.K. Human liver was obtained within 24 h of death from a 70-year-old male who died as a result of a myocardial infarct. The subject had no history of psychiatric illness. The liver was kindly supplied by Dr. G. Stirling, Department of Pathology, University of Nottingham, U.K.

Materials

NAD⁺ was purchased from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K. Tyramine hydrochloride, benzylamine, 5-hydroxytryptamine (creatinine sulphate complex), diaminobenzylamine hydrochloride, 1,4-diaminobutane and Coomassie Brilliant Blue R were purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey,

U.K. 2-Mercaptoethanol, sodium dodecyl sulphate, dimethylformamide, *N*-methylpyrrolidone, *NN'*-methylenebisacrylamide, acrylamide, *NNN'*-tetramethylenediamine, riboflavin and spheroidal hydroxyapatite were purchased from BDH Chemicals, Poole, Dorset, U.K. CNBr was purchased from Hopkin and Williams Ltd., P.O. Box 1, Romford RM1 1HA, Essex. *p*-Nitrobenzoyl azide was purchased from Eastman (Kodak Ltd), Kirkby, Liverpool L33 7UF, U.K. Glycerol (B.P. grade) was purchased from Hills Pharmaceuticals, Briercliffe, Burnley BB10 2JY, Lancs., U.K. Triton X-100 was purchased from Lennig Chemicals Ltd., Croydon, London CB9 3NB, U.K. Sepharose 4B and 6B were purchased from Pharmacia (G.B.) Ltd., London W.5, U.K. DEAE-cellulose (Whatman grade DE-52) was obtained from W. and R. Balston (Modified Cellulose) Ltd., Maidstone, Kent, U.K. Bio-Beads SM-2 (20–50 mesh) were purchased from Bio-Rad Laboratories Ltd., 27 Homesdale Road, Bromley, Kent, U.K. The Amicom Ultrafiltrator was obtained from Amicon, High Wycombe, Bucks., U.K. Conductivity measurements were made with a Radiometer Conductivity meter type CDM-2d. The MSE 'Atomix' was purchased from MSE Scientific Instruments, Manor Royal, Crawley, West Sussex, U.K. Benzylamine hydrochloride was prepared by treating the base, dissolved in diethyl ether, with conc. HCl. The filtered precipitate was recrystallized twice from acetone/water (10:1, v/v). Freund's adjuvant was obtained from Difco Laboratories, Detroit, MI, U.S.A. All other chemicals were of A.R. grade.

Aldehyde dehydrogenase

Ox liver aldehyde dehydrogenase was prepared by the method of Houslay & Tipton (1973).

Assay of monoamine oxidase

Monoamine oxidase was assayed by the coupled spectrophotometric method of Houslay & Tipton (1973) with ox liver aldehyde dehydrogenase. The assays were carried out at 30°C in plastic cuvettes with a 1 cm light-path and contained, in a final volume of 1 ml, 20 mM-potassium phosphate, pH 7.2, aldehyde dehydrogenase (0.1 μ mol/min), 0.45 mM-NAD⁺, 2.3 mM-tyramine hydrochloride and enzyme (20–300 μ g of protein). The reaction was started by the addition of substrate and the change in A_{340} was measured. Where indicated benzylamine hydrochloride (final concn. 2.3 mM) or 5-hydroxytryptamine (final concn. 1.1 mM) was used as substrate.

Fractions eluted from chromatographic columns were more conveniently assayed by a modification of the method of Tabakoff & Alivisatos (1972) using *p*-dimethylaminobenzylamine as substrate. Assays contained, in a final volume of 1.2 ml, 100 mM-

potassium phosphate, pH 7.2, 2.5 mM-*p*-dimethylaminobenzylamine and enzyme (20–300 μ g of protein). The samples were incubated at 30°C for 1 h and the A_{355} was determined. The reaction was linear with time for at least 1 h.

Protein determination

Protein at high concentrations (5–30 mg/ml) was determined by the method of Gornall *et al.* (1949) and at lower concentrations by the method of Wang & Smith (1975).

Preparation of butyl-tyramine-Sepharose

Butyl-tyramine-Sepharose was prepared by a modification of the method of Cuatrecasas (1970). Sepharose was activated with CNBr by the method of Porath *et al.* (1973). Sepharose 4B (50 ml) was washed extensively with water in a sintered-glass funnel by gentle suction. The Sepharose was suspended in 50 ml of 5 M-potassium phosphate buffer, pH 11.4 at 5°C, prepared by dissolving 3.33 mol of K₃PO₄ and 1.67 mol of K₂HPO₄/litre. CNBr (4 g) was dissolved in 20 ml of *N*-methylpyrrolidone/water (1:4, v/v). This solution was added to the Sepharose suspension and the mixture was gently stirred for 10 min at 5–10°C. The suspension was then extensively washed with ice-cold water on a sintered-glass funnel. The Sepharose was added to 100 ml of 2 M-1,4-diaminobutane (previously adjusted to pH 10 with 6 M-HCl) and gently shaken overnight at 4°C. The aminobutyl-Sepharose was suspended in 60 ml of 0.2 M-sodium borate buffer, pH 9.3, and 0.175 M-*p*-nitrobenzoylazide in dimethylformamide (40 ml) was added. The mixture was stirred for 1 h at room temperature (20°C). The *p*-nitrobenzamidobutyl-Sepharose was washed with 1 litre of dimethylformamide/water (1:1, v/v) and then suspended in 60 ml of 0.1 M-sodium dithionite in 0.5 M-NaHCO₃, pH 8.5, for 1 h at 40°C. The *p*-aminobenzamidobutyl-Sepharose was washed thoroughly with water, cooled to 4°C, and added to 200 ml of 0.1 M-NaNO₂ in 0.5 M-HCl at 4°C. The mixture was stirred for 7 min at 4°C and then washed with 500 ml of ice-cold 1% (w/v) urea followed by rapid washing with 2 litres of ice-cold water. The diazonium-Sepharose was then immediately added to 50 ml of 10 mM-tyramine hydrochloride in 0.2 M-sodium borate buffer, pH 10, and gently shaken at 4°C overnight. After coupling, the orange butyl-tyramine-Sepharose was washed extensively with water at room temperature and stored in water containing a few drops of toluene at 4°C. Before use columns of butyl-tyramine-Sepharose were washed with 100 ml of 1 M-KCl in 10 mM-potassium phosphate buffer, pH 7.2, containing 1.5% (w/v) Triton X-100 and 20% (v/v) glycerol and subsequently equilibrated in the same buffer without KCl.

Purification of monoamine oxidase

All operations were carried out at 4°C unless otherwise stated.

Preparation of Triton X-100 extracts of mitochondria. Mitochondrial preparations were obtained from 100 g (fresh wt.) of rat or human liver by the method of Hawkins (1952) and washed once in 0.25M-sucrose (previously adjusted to pH 7.0 with 0.5M-NaHCO₃). Rat liver was homogenized with a Potter-Elvehjem homogenizer whereas the more fibrous human liver required homogenization for 1 min in a MSE Atomix homogenizer. The washed mitochondrial fractions were resuspended in 20mM-potassium phosphate buffer, pH 7.2, to give a final volume of 100 ml and stored frozen at -15°C for at least 48 h. The mitochondrial preparations were slowly thawed and then centrifuged for 60 min at 100000g_{av.}. The pellet was resuspended by gentle homogenization in 20mM-potassium phosphate buffer, pH 7.2, containing 1.5% (w/v) Triton X-100 (100 ml) and the mixture was stirred for 1.5 h. The suspension was then centrifuged for 1.5 h at 100000g_{av.} and the supernatant used as the source of the enzyme.

Removal of Triton X-100 and (NH₄)₂SO₄ precipitation. Triton X-100 was removed from the supernatant by means of Bio-Beads SM-2 previously equilibrated in 20mM-potassium phosphate buffer, pH 7.2, by the method of Holloway (1973). The Triton X-100 extract (100 ml) was stirred at 0°C and approx. 100 g (dry wt.) of Bio-Beads were added in 10 g portions over a period of 1–2 h until the A₂₈₀/A₂₆₀ ratio of the supernatant remained constant. The suspension was filtered by gentle suction on a sintered-glass funnel and the Bio-Beads were carefully washed with 100 ml of ice-cold 20mM-potassium phosphate buffer, pH 7.2. The filtrate and washings were combined and solid (NH₄)₂SO₄ was added slowly, with stirring at 0°C, to give a concentration of 361 g/litre of initial volume. The solution was maintained at pH 7.2 by the addition of 0.5M-KOH. The solution was stirred for 30 min and the precipitate collected by centrifugation for 20 min at 25000g_{av.}. The supernatant was discarded and the precipitate suspended in 100 ml of 20mM-potassium phosphate buffer, pH 7.2, and dialysed overnight against 50 vol. of the same buffer. The human liver preparation remained insoluble at this stage of purification but its activity was not impaired.

Chromatography on DEAE-cellulose. Each dialysed preparation was diluted to 200 ml with 20mM-potassium phosphate buffer, pH 7.2, and applied to a column (2.5 cm × 20 cm) of DEAE-cellulose previously equilibrated in the same buffer. The columns were washed with 100 ml of the buffer.

(a) Elution of monoamine oxidase of rat liver. Monoamine oxidase from rat liver was eluted with approx. 200 ml of the same buffer containing 0.2% (w/v) Triton X-100. Solid (NH₄)₂SO₄ was added to

the eluate slowly, with stirring at 0°C to give a concentration of 361 g/litre of initial volume. The solution was maintained at pH 7.2 by the addition of 0.5M-KOH. The solution was stirred for 30 min and the precipitate collected by centrifugation for 20 min at 25000g_{av.}. The pale-yellow floating precipitate was carefully removed and dissolved in 5 ml of 20mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol and 1.5% (w/v) Triton X-100. The solution was dialysed overnight against 50 vol. of the same buffer.

(b) Elution of monoamine oxidase of human liver. Monoamine oxidase from human liver was eluted by means of a linear gradient (200 ml) of Triton X-100 (0–1%, w/v) in 20mM-potassium phosphate buffer, pH 7.2, containing 0.2M-KCl. Fractions containing monoamine oxidase activity were pooled and concentrated with an Amicon Ultrafiltrator with a PM10 membrane. The concentrated solution (5 ml) was subsequently dialysed overnight against 50 vol. of 20mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol and 1.5% (w/v) Triton X-100.

Chromatography on Sepharose 6B. Each dialysed preparation was chromatographed on a column (3 cm × 80 cm) of Sepharose 6B equilibrated in 20mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol and 1.5% (w/v) Triton X-100. The fractions with the highest specific activity were pooled and dialysed overnight against 50 vol. of 10mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol.

Chromatography on hydroxyapatite. Each dialysed preparation was applied to a column (2 cm × 10 cm) of spheroidal hydroxyapatite equilibrated in 10mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol. The column was washed with 50 ml of the same buffer.

(a) Elution of monoamine oxidase of rat liver. Monoamine oxidase from rat liver was eluted in two phases: (i) by applying 50 ml of 200mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol; (ii) by subsequently applying 50 ml of the same buffer containing 0.2% (w/v) Triton X-100. The second fraction had the highest specific activity and was used in the next purification step.

(b) Elution of monoamine oxidase of human liver. Monoamine oxidase from human liver was eluted from the column by 50 ml of 200mM-potassium phosphate buffer, pH 7.2, containing 0.1 mM-mercaptoethanol. No further activity could be eluted by adding Triton X-100 to the buffer.

The preparations of monoamine oxidase from rat or human liver were dialysed overnight against 50 vol. of 10mM-potassium phosphate buffer, pH 7.2 containing 1.5% (w/v) Triton X-100 and 20% (v/v) glycerol.

Chromatography on butyl-tyramine-Sepharose. The

dialysed preparations were applied to columns (2cm×10cm) of butyl-tyramine-Sepharose equilibrated in 10mM-potassium phosphate buffer, pH 7.2, containing 1.5% (w/v) Triton X-100 and 20% (v/v) glycerol. The columns were washed with 2–3 column volumes of the same buffer and monoamine oxidase activity was eluted by applying a linear gradient (40ml) of KCl (0–0.2M) in the same buffer.

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. Polyacrylamide disc gel electrophoresis of protein samples, and subsequent staining for protein was performed by the method of Betts & Mayer (1975) in 5% (w/v) or 10% (w/v) polyacrylamide gels.

Preparation of antisera to monoamine oxidase from rat or human liver

Samples of monoamine oxidase were dialysed overnight against water, freeze-dried and dissolved in water (12ml). Antisera were raised in sheep by injecting 240 µg of purified enzyme with Freund's complete adjuvant on four occasions. Antigen was injected at six sites (four subcutaneously and two intramuscularly) on each occasion at 14-day intervals. The volume injected at each site was 1 ml (0.5 ml of antigen solution and 0.5 ml of adjuvant). At 1 week after the final injection the animal was bled by jugular cannulation. The blood was left to clot and centrifuged at 1500g_{av.} for 30 min. An equal volume of neutralized satd. (NH₄)₂SO₄ was added to the serum at 0°C and after stirring for 30 min the precipitate was collected by centrifugation at 25000g_{av.} for 20 min. The precipitate was washed twice with (NH₄)₂SO₄ solution (291 g/litre) at 0°C, dissolved in a volume of 10mM-potassium phosphate buffer, pH 8.0, equal to one-half the initial serum volume and dialysed overnight against 5 litres of the same buffer. The suspension was subsequently centrifuged at 25000g_{av.} for 20 min. The supernatant was dialysed overnight against 5 litres of 20mM-sodium phosphate, pH 7.0, containing 0.15M-NaCl. The preparation was

subsequently heated at 70°C for 30 min, centrifuged at 25000g_{av.} for 20 min, and the supernatant was stored frozen at –15°C.

Immunotitrations

Triton X-100 extracts of mitochondrial preparations from rat and human liver were depleted of micellar Triton X-100 with Bio-Beads SM-2 as described above. Immunotitrations were carried out with antisera or control sera that had been processed as described above. Samples (0.2–0.3 ml) of Triton X-100-depleted extract were mixed with processed sera and adjusted to a final volume of 1 ml with 20mM-sodium phosphate, pH 7.0, containing 0.15M-NaCl. The solutions were kept at 4°C overnight and then centrifuged at 14000g_{av.} for 6 min. Samples (0.1–0.25 ml) of the supernatants were assayed for monoamine oxidase activity with 5-hydroxytryptamine, tyramine and benzylamine. Immunoprecipitates were washed twice with 20mM-sodium phosphate, pH 7.0, containing 0.15M-NaCl and protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Experimental and Results

Monoamine oxidase from rat and human liver was purified to homogeneity by the criterion of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. Typical purification schemes for the two enzymes are shown in Tables 1 and 2. The purified monoamine oxidase from rat liver had a specific activity of 16.1 nmol/min per mg of protein when assayed with tyramine although the eluate from the Sepharose 6B column had a higher specific activity of 41.2 nmol/min per mg of protein. The specific activity of monoamine oxidase from rat liver declined after chromatography on hydroxyapatite and butyl-tyramine-Sepharose. The preparation of monoamine oxidase from human liver had a final specific activity of 12.5 nmol/min per mg of

Table 1. *Purification of monoamine oxidase from mitochondrial preparations of rat liver*
Except where indicated all assays were carried out with tyramine as substrate.

	Vol. (ml)	Protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg of protein)	Yield	Purification (fold)	Relative enzyme activities (tyramine/ 5-hydroxytryptamine/ benzylamine)
Mitochondrial preparation	100	1284	1139	0.88	(100)	—	1:0.23:0.47
Triton X-100 extract	100	412	828	2.0	72.6	2.3	1:0.20:0.50
DEAE-cellulose eluate	185	45	642	14.2	56.3	16.1	—
Sepharose 6B eluate	60	10.5	433	41.2	38.0	46.8	—
Hydroxyapatite eluate	10	5.6	125	22.3	10.9	25.3	—
Butyl-tyramine-Sepharose eluate	12	2.3	38	16.1	3.3	18.3	1:0:0.57

Table 2. *Purification of monoamine oxidase from mitochondrial preparations of human liver*
Except where indicated all assays were carried out with tyramine as substrate.

	Vol. (ml)	Protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg of protein)	Yield	Purification (fold)	Relative enzyme activities (tyramine/ 5-hydroxytryptamine/ benzylamine)
Mitochondrial preparation	100	840	257	0.3	(100)	—	1:0.35:0.70
Triton X-100 extract	100	200	282	1.4	109	4.7	1:0.35:0.53
DEAE-cellulose eluate	80	75	88	1.2	34.2	3.9	—
Sephacrose 6B eluate	48	21	49	2.3	19.0	7.7	—
Hydroxyapatite eluate	14	7.2	40	5.5	15.5	18.3	—
Butyl-tyramine-Sephacrose eluate	10	1.75	22	12.5	8.5	41.6	1:0:0.90

Fig. 1. *Immunotitration of monoamine oxidase activities in extracts of mitochondrial preparations from rat liver* Samples (0.2 ml) of Triton X-100-depleted extracts of mitochondrial preparations were incubated overnight at 4°C with antiserum. The mixtures were centrifuged at 14000g_{av} for 6 min and enzyme activities assayed in the supernatants. Enzyme activity was assayed with tyramine (●), 5-hydroxytryptamine (■) and benzylamine (○). ▲, Protein in the immunoprecipitates.

protein when assayed with tyramine and was purified 41.6-fold with respect to the mitochondrial preparation. Although the enzyme from human liver had a lower final specific activity than the enzyme from rat liver it was recovered in higher yield. Both purified preparations did not oxidize 5-hydroxytryptamine and slight increases (approx. 1.25-fold) in the relative activity of the purified enzymes towards benzylamine were observed compared with the relative activity of the enzymes in mitochondrial preparations.

Polyacrylamide-gel electrophoresis of samples of purified monoamine oxidase (20–100 μg of protein) in the presence of sodium dodecyl sulphate showed one band which stained for protein. In some cases this stained band was coincident with a faint yellow band that was observed in unstained gels (results not shown).

The use of Bio-Beads SM-2 was very effective in removing micellar Triton X-100. In preliminary experiments (NH₄)₂SO₄ fractionation of Triton X-100 extracts of mitochondrial preparations (0–30% satn.) gave a floating precipitate which contained approx. 40% of the enzyme activity. Further (NH₄)₂SO₄ fractionation (30–60% satn.) gave a sedimenting precipitate that contained the remainder of the enzyme activity. Further work showed that the first (NH₄)₂SO₄ fractionation (0–30% satn.) precipitated Triton X-100 from the extract which appeared in the floating layer. Treatment of Triton X-100 extracts with Bio-Beads SM-2 removed micellar Triton X-100 and subsequent (NH₄)₂SO₄ fractionation (0–60% satn.) and centrifugation gave a sedimenting precipitate. This treatment avoids the loss of enzyme activity associated with the first (NH₄)₂SO₄ fractionation (0–30% satn.) and facilitated the subsequent binding of the enzyme to DEAE-cellulose. The monoamine oxidase activity of human liver was rendered insoluble by these procedures but this did not impair its enzyme activity.

Significant differences were noted in the behaviour of the two enzymes when chromatographed on DEAE-cellulose and hydroxyapatite. The monoamine oxidase from human liver was bound more strongly to DEAE-cellulose and therefore a higher salt and detergent concentration was required for the elution of the enzyme. However, monoamine oxidase from human liver bound less strongly to hydroxyapatite than the enzyme from rat liver. It was therefore eluted by 200 mM-potassium phosphate buffer, pH 7.2, without the addition of detergent. The two enzymes behaved identically on butyl-tyramine-Sephacrose.

The results in Fig. 1 show that the enzyme activities with 5-hydroxytryptamine, tyramine and benzylamine are completely immunoprecipitated from Triton X-100-depleted extracts of mitochondrial preparations of rat liver by the same volume of processed antiserum. Similar results were obtained with mitochondrial extracts from human liver (Fig. 2).

Fig. 2. Immunotitration of monoamine oxidase activities in extracts of mitochondrial preparations from human liver. Samples (0.3 ml) of Triton X-100-depleted extracts of mitochondrial preparations were incubated overnight at 4°C with antiserum. The mixtures were centrifuged at 14000*g*_{av} for 6 min and enzyme activities assayed in the supernatants. Enzyme activity was assayed with tyramine (●), 5-hydroxytryptamine (■) and benzylamine (○). ▲, Protein in the immunoprecipitates.

Incomplete precipitation of monoamine oxidase activity occurred when extracts of mitochondrial preparations in 1.5% (w/v) Triton X-100 were treated with antiserum. This observation agrees with that of Hartman *et al.* (1971). The removal of micellar Triton X-100 with Bio-Beads SM-2 resulted in the complete immunoprecipitation of monoamine oxidase activity. Monoamine oxidase was not precipitated with control sera.

Discussion

The purification scheme for monoamine oxidase described here combines the classical purification procedures with a novel 'affinity' chromatographic step using diazo-coupled tyramine-Sepharose. Baket & Hemsworth (1975*a,b*) have used inhibitors of monoamine oxidase as affinity ligands and have achieved a fourfold purification of the enzyme with a hydrazine-Sepharose column and a 40-fold (1% yield) purification with a 1-*m*-aminophenyl-2-cyclopropylaminoethanol-Sepharose column from extracts prepared by sonicating mitochondrial preparations. More recently Toraya *et al.* (1976) purified amine oxidase (ninefold, 32% yield) from extracts of *Aspergillus niger* by chromatography on a column of aminohexyl-Sepharose, a matrix that failed to purify monoamine oxidase from rat liver (Dennick & Mayer, 1976).

Previous work (Dennick & Mayer, 1976) has shown that monoamine oxidase can be purified 20-fold and in 50% yield from Triton X-100 extracts of mitochondrial preparations from rat liver by chromatography on butyl-tyramine-Sepharose. The

purification achieved with this system was critically dependent on the chain length of the spacer arm and the ionic strength of the equilibrating buffer. A butyl spacer arm, 10 mM-potassium phosphate buffer, pH 7.2, and the presence of 20% (v/v) glycerol were necessary for binding and subsequent elution from the column with KCl. It is noteworthy that Aunis *et al.* (1973) prepared highly purified dopamine β -hydroxylase (EC 1.14.17.1) using ethyl-tyramine-Sepharose as an affinity matrix. These workers found that the enzyme was simply retarded on the affinity column and that washing with equilibrating buffer was sufficient to achieve considerable purification.

It is unlikely that the purification of monoamine oxidase from rat and human liver on butyl-tyramine-Sepharose is due to affinity chromatography. Substrate fails to elute the enzyme and protein remains bound to the column even after washing with 0.5 M-KCl. This suggests that the purification is possibly due to non-specific hydrophobic and/or ionic interactions. The terms 'detergent' (Wilchek, 1974) or 'apolar' (Phan & Mahler, 1976) chromatography have been applied to such systems.

The use of Bio-Beads SM-2 to remove micellar Triton X-100 from enzyme preparations had several advantages. The binding of monoamine oxidase to DEAE-cellulose is critically dependent on the concentration of Triton X-100. As little as 0.075% (w/v) Triton X-100 can prevent binding to DEAE-cellulose (Houslay & Tipton, 1975). Other workers have attempted to remove Triton X-100 by fractionation with $(\text{NH}_4)_2\text{SO}_4$, which at a concentration of 27% satn. precipitates micellar Triton X-100 (R. G. Dennick & R. J. Mayer, unpublished observation). Thus Youdim & Collins (1971), Houslay & Tipton (1973) and Youdim (1976) have used an initial $(\text{NH}_4)_2\text{SO}_4$ fractionation step (0–30% satn.). This procedure was not used in the work described here since it involves discarding a considerable fraction (40%) of monoamine oxidase activity. After removing micellar Triton X-100 with Bio-Beads SM-2 followed by $(\text{NH}_4)_2\text{SO}_4$ fractionation (0–60% satn.) and dialysis against detergent-free buffer, monoamine oxidase was recovered in high yield and subsequently bound easily to DEAE-cellulose.

The differences in behaviour of the enzymes from rat and human liver during chromatography on DEAE-cellulose and hydroxyapatite are difficult to explain. The human liver enzyme preparation was obtained by more vigorous homogenization than the enzyme preparation from rat liver and contained a considerable quantity of fatty material. After removal of Triton X-100 and $(\text{NH}_4)_2\text{SO}_4$ fractionation the enzyme preparation from human liver was insoluble and was applied to the DEAE-cellulose column as a fine suspension. This may account for the conditions that were needed to elute the enzyme from the DEAE-

cellulose column and the concomitant slight decrease in specific activity. The behaviour of monoamine oxidase from rat liver during chromatography on hydroxyapatite is similar to that reported for the enzyme from ox liver (Yasunobu *et al.*, 1968; Gomes *et al.*, 1969). These workers obtained two fractions with monoamine oxidase activity. Fraction C1 was obtained by eluting the column of hydroxyapatite with 0.2M-potassium phosphate buffer, pH7.4, and fraction C2 was obtained by elution with 0.2M-potassium phosphate buffer, pH7.4, containing 0.15% (w/v) sodium deoxycholate. Fraction C2 had the highest specific activity and was shown to have a mol.wt. of 120000 whereas fraction C1 had a mol.wt. of 400000. However, Hartman *et al.* (1971) showed that fractions C1 and C2 were immunologically identical and suggested that fraction C2 was a trimer of fraction C1 representing a larger fragment from the outer membrane of the mitochondrion. In the work reported here monoamine oxidase from rat liver was eluted from hydroxyapatite by 0.2M-potassium phosphate buffer, pH7.2. Subsequently a fraction of higher specific activity could be obtained by elution of the column with 0.2M-potassium phosphate buffer, pH7.2, containing 0.2% (w/v) Triton X-100.

Several groups of workers have raised antisera to monoamine oxidase from bovine (Hidaka *et al.*, 1971; Hartman *et al.*, 1971; McCauley & Racker, 1973) and rat (Youdim & Collins, 1975) liver. The antisera have been used in the qualitative (Youdim & Collins, 1975) and quantitative (Hidaka *et al.*, 1971; Hartman *et al.*, 1971) assessment of the nature of liver monoamine oxidase. The data have been interpreted to mean that liver monoamine oxidase is assembled from the same protein subunit into forms of different molecular weight (Hartman *et al.*, 1971) or that two distinct forms of liver monoamine oxidase exist (Youdim & Collins, 1975).

The results in Figs. 1 and 2 show that monoamine oxidase is quantitatively precipitated from Triton X-100-depleted extracts of mitochondrial preparations from rat and human liver. The volume of antiserum required to completely precipitate enzyme activity is the same when 5-hydroxytryptamine, benzylamine or tyramine is used as substrate. The results indicate that these enzyme activities are associated with a single immunogenic macromolecular species and consequently are completely precipitated from the mitochondrial extracts by an identical volume of antiserum.

The results show that the functional forms of monoamine oxidase which may be resolved by pharmacological (Johnston, 1968) or kinetic (Houslay & Tipton, 1974) means are associated with a single macromolecular species. The data would support the contention of Houslay & Tipton (1973) that the multiple forms of monoamine oxidase activity are

associated with a single protein species which is modified by differential binding of phospholipid.

The purification procedure for the enzymes described in the present paper gives homogeneous preparations of monoamine oxidase by the criterion of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. The enzyme preparations from both rat and human liver are not active against 5-hydroxytryptamine but the antisera against these antigens immunoprecipitate enzyme activity of the so-called A and B forms of the enzyme (5-hydroxytryptamine and benzylamine respectively) and activity characteristic of both forms (tyramine). The results show that a specific B form of the enzyme was not purified and that the different enzyme activities reside with a common antigenic species.

It is now possible to study immunochemically the synthesis and degradation of the enzyme in rat liver in different physiological states.

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