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Purification and Partial Characterization of Mammalian Cu-Dependent Amine Oxidases

Gunnar Houen,[a,*] Jytte Jørgensen,[b] Lise Leonardsen[b] and Lars-Inge Larsson[b]

[a]Institute of Biochemical Genetics, University of Copenhagen, Ø. Farimagsgade 2A, DK-1353 Copenhagen K., Denmark and [b]Department of Molecular Cell Biology, Division of Biotechnology, State Serum Institute, Building 81, Amager Boulevard 80, DK-2300 Copenhagen S., Denmark

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Bovine serum amine oxidase, porcine kidney diamine oxidase and human placental and pregnancy serum diamine oxidases have been purified by affinity chromatography and ion exchange chromatography. The purified enzymes were subjected to peptide mapping studies with trypsin, *S. aureus* V8 protease and pepsin. These studies revealed similarities between the enzymes and partial sequences from the bovine serum amine oxidase were obtained. The sequences obtained showed no homology to known sequences. Immunological studies using monoclonal antibodies to the purified enzymes revealed cross reactivity between the four enzymes. These results support the view that the Cu-dependent amine oxidases constitute a closely related group (E.C. 1.4.3.6).

Current evidence indicates that mammalian amine oxidases can be divided into two groups according to the nature of the prosthetic group: FAD-dependent (E.C. 1.4.3.4) (monoamine oxidases A and B and polyamine oxidase) and Cu-dependent (E.C. 1.4.3.6), diamine oxidase = histaminase, serum amine oxidase, and lysyl oxidase.[1] While the FAD-dependent mitochondrial monoamine oxidases have been reasonably well characterized,[2–4] the intracellular FAD-dependent polyamine oxidase remains less well characterized, apart from its having a relative molecular weight (M_r) of 60000 corresponding to that of the monoamine oxidases.[5,6]

The Cu-dependent amine oxidases form an even less well characterized group. This seems to be due partly to the fact that these enzymes have overlapping substrate specificities and are found in both extracellular and intracellular forms and partly to the difficulties experienced in the characterization of the organic prosthetic group.[1,7–9]

The nomenclature reflects this situation and the enzymes are named partly according to the tissue of origin and partly according to substrate specificity.[1] The best characterized enzymes are porcine kidney diamine oxidase (PKDAO), which shows high activity towards putrescine, and bovine serum amine oxidase (BSAO), which shows high activity towards benzylamine and polyamines.[1,10–12] In addition to these, human pregnancy serum contains an amine oxidase (HPSDAO) with activity towards putrescine, spermidine and spermine,[13] human placenta contains high amounts of diamine oxidase (HPDAO)[14] and the intestine is generally known to contain high amounts of diamine oxidase.[1,10,15] In an attempt to characterize this group of enzymes in more detail we have purified amine oxidases from bovine serum, porcine kidney, human pregnancy serum and human placenta. The purified enzymes were used for peptide mapping studies and for raising monoclonal antibodies. These studies all showed partial identity between the enzymes from these sources.

Materials and methods

Chemicals. All chemicals used were of analytical grade or higher quality. NaCl, Na_2HPO_4, NaH_2PO_4, H_3PO_4, triethylamine, methyl α-mannopyranoside, ammonium peroxodisulfate, H_2O_2 (30%), glycerol, glycine, MeOH, CH_3COOH, CH_3CN, and toluene were from Merck (Darmstandt, Germany). Benzylamine, putrescine · 2HCl, spermidine · 3HCl, spermine · 4HCl, horseradish peroxidase, sodium dodecyl sulfate, dithiothreitol, tris(hydroxymethylamino)methane (TRIS), dansyl chloride, trypsin, pepsin, and electrophoresis low molecular weight standards were from Sigma (St. Louis, USA). Acrylamide, bisacrylamide, tetramethylethylenediamine and hydroxylapatite were from Bio-Rad (Richmond, USA).

Staphylococcus aureus V8 enzyme was from Miles (Naperville, USA), AH-Sepharose, ConA-Sepharose, and mono Q columns were from Pharmacia (Uppsala,

* To whom correspondence should be adressed.

Sweden). [2,3-^{3}H]Putrescine was from New England Nuclear (Boston, USA). *o*-Phenylenediamine (OPD) was from Kem-En-Tec (Copenhagen, Denmark). DEAE cellulose was from Whatman (Maidstone, UK). Scintillation fluid was from J.T. Baker (Deventer, Holland). Trifluoroacetic acid (TFA) was from Applied Biosystems (Foster City, USA). Fetal calf serum was from Gibco (Paisley, UK). Bovine serum was from *Statens Serum Institut* (Copenhagen, Denmark), bovine blood from *Roskilde forsøgsslagteri* (Roskilde, Denmark) and human pregnancy sera and placentas from *Rigshospitalet* (Copenhagen, Denmark).

Gel electrophoresis. SDS-PAGE was done by the method of Laemmli.[16] Gels were of 0.75 mm thickness and were cast and run using a 'Mighty small' apparatus (Hoefer Scientific, San Francisco, USA).

Amino acid analysis. Samples for amino acid analysis were hydrolysed in 6 M HCl containing 0.5% phenol for 24 h at 110°C. After drying, the samples were analysed on an ion-exchange column using a Waters HPLC system with post-column OPA quantitation of amino acids as described.[17]

Amino acid sequence analysis. Protein sequence analysis was done on a Model 477A Sequenator (Applied Biosystems, Foster City, CA, USA) with on-line analysis of phenylthiohydantoin (PTH) amino acids.

HPLC analysis of polyamines. Samples for HPLC were dansylated and analysed as described by Brossat *et al.*[18] using a linear gradient from 50% MeOH, 50% 50 mM triethylammonium phosphate pH 4 to 100% MeOH over 15 min (A) or from 60% MeOH to 100% MeOH over 30 min (B). HPLC was done on a Waters system with two model 510 pumps, a model 420-AC fluorescence detector, a 710 B WISP, a Waters Data Module, a Z-module with a 8 mm × 10 cm RP-8 column, and a Waters System Controller.

Peptide mapping. Samples of 100 µl (1 mg ml^{-1}) were incubated at 37°C or at room temperature with trypsin, 1 : 100 (w/w), *S. aureus* V8, 1 : 100 (w/w) or pepsin, 1 : 100 (w/w). Reactions were terminated by addition of 10 µl 10% TFA. Samples were then analysed on a model 130 A HPLC with a 2.1 mm × 220 mm RP-18 column (5 µm particle size, Applied Biosystems, Foster City, USA), using a linear gradient of CH_3CN in 0.1% TFA: flow, 275 µl min^{-1}; column temp., 35°C; detection, 220 nm. V8 digests were analysed on a Waters HPLC (described above) using a 5 mm × 100 mm RP18-column (10 µm particles) (Waters Novapak): flow, 500 µl min^{-1}; column temp., 20°C; detection, 220 nm.

Enzyme assays. Benzylamine oxidase activity was determined as described by Tabor *et al.*[19] using 1 mM benzylamine in 0.1 M phosphate buffer (PB) pH 7.2 as the substrate. One hundred µl of fractions to be assayed were added to 1 ml of substrate and samples were incubated at 37°C for 2–16 h. The change in absorption at 250 nm was measured and converted into activity using $E_{250} = 12.500\ M^{-1}\ cm^{-1}$ for benzaldehyde.[20] One unit is defined as 1 µmol min^{-1} mg^{-1}. Putrescine oxidase activity was determined by the method of Okuyama and Kobayashi[21] using 1 mM putrescine with 50 µCi [2,3-^{3}H] putrescine per ml in M PB pH 7.2. One hundred µl substrate (2 mM) was mixed with 100 µl of sample and incubated at 37°C for 2–16 h. After incubation samples were extracted with 1 ml toluene, and after freezing on dry-ice the toluene phase was decanted into scintillation vials to which 4 ml scintillation fluid had been added. Samples were counted in a model LS 900 scintillation counter (Beckman). Alternatively putrescine oxidase activity was determined by a coupled peroxidase assay using 1 mM putrescine in 0.1 M PB, pH 7.2, with 10 µg HRP per ml and 4 mg OPD per 50 ml. One hundred µl of sample were incubated in 1 ml of the assay mixture at 37°C for 16 h and the reaction was terminated by addition of 1 ml of 1 M H_2SO_4. The absorption at 490 nm was measured and converted into activity using a standard curve for H_2O_2. Spermine oxidase activity was determined by incubating samples with 0.1 mM spermine in 0.1 M PB, pH 7.2, at 37°C for 2–16 h. The reaction was terminated by addition of perchloric acid and samples were dansylated and analysed by HPLC as described above.

Homogenization and extraction of placentas. One placenta was homogenized in 1 l 0.1 M PB, pH 7.2, for 15 min at 4°C, using an Ultra Turrax (Janke und Kunkel, Breisgau, Germany) homogenizer. The homogenate was centrifuged at 8000*g* to give 1.2 l of supernatant which was used immediately.

Ammonium sulfate precipitations. Ammonium sulfate (400 g l^{-1}) was added and the solution stirred overnight. The precipitate was isolated by centrifugation at 13000*g*, redissolved in 0.1 × the starting volume, and dialysed against 5 mM PB pH 7.2 (3 × 5 l). The dialysed solution was centrifuged before further use.

AH-Sepharose chromatography. The column (1 cm × 20 cm) was equilibrated in 5 mM PB, pH 7.2, and the sample (100–1000 ml) was pumped onto the column (0.2 ml min^{-1}). The column was then washed with 5 mM PB pH 7.2 until the absorption at 280 mm was below 0.05 and then eluted with a gradient of increasing PB concentration and finally eluted with 10 mM octylamine in PB pH 7.2.

ConA Sepharose chromatography. The column (1 cm × 10 cm) was equilibrated in 0.1 M PB pH 7.2. The sample (50–100 ml) was pumped onto the column, which was then washed with 0.1 M PB pH 7.2 until the absorbance at 280 nm was below 0.05. Bound proteins were eluted

with 0.3 M methyl α-mannopyranoside, 0.1 mM $CaCl_2$ in 0.1 M PB pH 7.2; flow, 0.2 ml min^{-1}.

Mono Q ion-exchange chromatography (FPLC). One ml samples were chromatographed on mono Q columns (1 ml) which were equilibrated in 50 mM PB pH 8.0 and eluted with a linear gradient to 1 M NaCl in 50 mM PB pH 8.0 over 30 min or 1 h; flow, 1 ml min^{-1}.

DEAE-cellulose chromatography. The column (1 cm × 30 cm) was equilibrated in 50 mM TRIS pH 8.0. After loading of the sample the column was washed and then eluted with a linear gradient to 1 M NaCl in 50 mM TRIS pH 8.0; flow, 0.2 ml min^{-1}.

Hydroxylapatite chromatography. The column (1 cm × 10 cm) was equilibrated in 10 mM PB pH 7.2. After loading of the sample, the column was washed with 10 mM PB pH 7.2 and then eluted with a linear gradient up to 0.5 M PB pH 7.2; flow, 0.2 ml min^{-1}.

Monoclonal antibodies: immunizations. 4–6-week-old BALB/c mice (Hvidsten, Hillerød, Denmark) were immunized intraperitoneally with BSAO or HPDAO. Each mouse received 50 μg antigen absorbed onto $Al(OH)_3$ gel (6.9 mg ml^{-1}; State Serum Institute, Copenhagen, Denmark) per immunization. The animals were immunized 2–4 times with 14 day intervals. 2–3 days before the fusion, a 50 μg booster injection was given intraperitoneally.

Fusions. Mouse myeloma cells (X63-Ag 8.653) were cultured in logarithmic phase in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum (normal medium) before fusion with splenocytes isolated from immunized mice. A number of fetal calf serum batches were initially screened for benzylamine and diamine oxidase activities and a particular batch (from Biocrom KG, Darmstadt, Germany) devoid of such enzyme activity was employed throughout. Fusions were carried out at room temperature. The fused cells were dispersed in normal medium supplemented with 13.6 μg ml^{-1} hypoxanthine, 4.5 μg ml^{-1} aminopterin and 7.6 μg ml^{-1} thymidine (HAT medium) into 12 96-well plates (NUNC) containing 1- to 2-day-old feeder cells (10^4 mouse peritoneal cells in 100 μl HAT medium per well). Plates were incubated at 37°C in air–5% CO_2 at 90% humidity. No medium replacement took place before the initial screening. Positive cultures were cloned by limiting dilution cloning. Antibody subclasses were determined using a Mono AB-ID™ Mouse EIA kit (Zymed, San Franciso, California, USA).

Ascites production. Ascites fluid was collected from pristane- (Aldrich, Steinheim, Germany) primed Balb C/CFI mice at about 2 weeks after intraperitoneal injection of hybridoma cells. Ascites fluids were dextran sulfate-precipitated before purification of the monoclonal antibodies on protein A Sepharose columns. Antibodies were eluted with 0.1 M citric acid, 0.5 M NaCl buffer pH 2.8, and neutralized with 1 M Tris buffer, pH 11.0. Purified antibodies were stored in aliquots at 4°C with 0.01% NaN_3. The purity of the antibodies was controlled by SDS-PAGE.

Immunopurification. Purified monoclonal antibodies were immobilized on divinylsulfonylagarose (Kem-En-Tek, Copenhagen) by mixing 1 ml antibody solution in 0.1 M Na_2CO_3, pH 9.0 (2 mg ml^{-1}) with 1 ml divinylsulfonylagarose which had been prewashed with 0.1 M Na_2CO_3, pH 9.0. After incubation overnight at 5°C, 0.2 ml of 1 M ethanolamine pH 9.0 was added and incubation continued for 2 h. The resulting resin was washed extensively with 0.1 M PB pH 7.2 before use. Samples (10 ml) were passed through the column which was then washed with 0.1 M PB pH 7.2 until $A_{280} = 0$, then washed with 10 ml of 0.1 M PB pH 7.2, 0.3 M NaCl and again with 10 ml 0.1 M PB. Finally bound proteins were eluted with 4 ml of 1 M CH_3COOH. The eluate was freeze-dried and redissolved in 0.1 M PB pH 7.2 (1 ml).

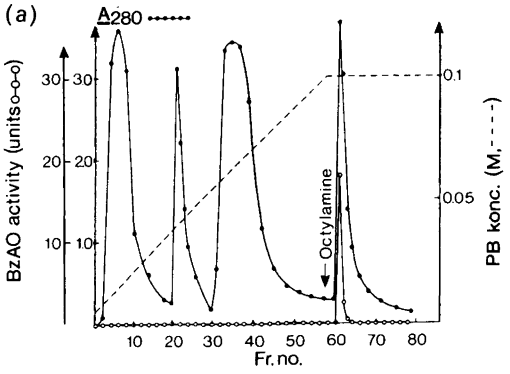
Western blots. Samples were subjected to SDS-PAGE and electrotransferred to polyvinyl difluoride membranes using 25 mM TRIS, 0.2 M glycine pH 8.3 as the transfer buffer (0.1 mA cm^{-2}, 16 h). Membranes were blocked in skimmed milk and incubated overnight at 5°C with antibodies diluted 1 : 500 in skimmed milk. Incubation was continued for 1 h at room temperature and membranes were then washed with skimmed milk 3 × 15 min and incubated 2 h with peroxidase-conjugated rabbit anti-mouse antiserum (1 : 500 in skimmed milk) (Dakopatts, Copenhagen). After 3 × 15 min washes in skimmed milk and one wash in 50 mM NaAc pH 5.0 membranes were incubated with 50 ml 50 mM NaAc pH 5.0 containing 1 ml N,N-dimethylformamide in which 10 mg 3-amino-9-ethylcarbazole had been dissolved.

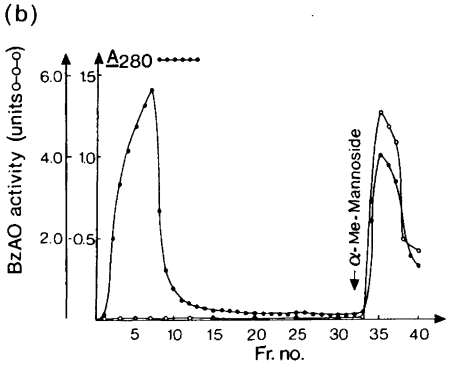
Dot blots. 10 μl samples were spotted on polyvinyldifluoride membranes which were then dried, wetted with MeOH, blocked with skimmed milk and then incubated with antibodies as described for Western blots above.

Results

Bovine serum amine oxidase was purified essentially as described[12,20] by ammonium sulfate precipitation followed by affinity chromatography on aminohexyl-Sepharose and ConA Sepharose, with additional final chromatography on a mono Q ion exchange column (Fig. 1). The specific activity of the purified enzyme was 0.2 units mg^{-1} using benzylamine as the substrate. The purified enzyme had a monomer M_r of 90 kDa and a dimer M_r of 180 kDa (Fig. 2). Occasionally higher M_r oligomers were also observed.

In the same way porcine kidney diamine oxidase,





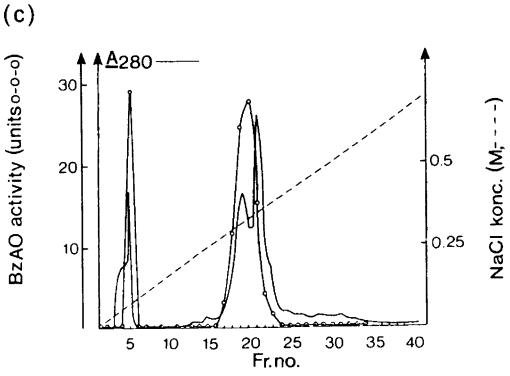


Fig. 1. (a) Chromatography of 100 ml bovine serum on AH-Sepharose. The sample was applied to the column (1 cm x 20 cm) which was washed in 5 mM PB, pH 7.2 eluted with a linear gradient from 5 mM PB, pH 7.2 to 100 mM PB, pH 7.2 and finally eluted with 100 mM PB, pH 7.2–10 mM octylamine (arrow); flow: 0.5 ml min^{-1}. Ten ml fractions were collected and assayed for benzylamine oxidase activity. (b) Chromatography of pooled active fractions from AH-sepharose chromatography on ConA sepharose. Thirty ml were applied to a 5 ml column which was then washed with 0.1 M PB, pH 7.2 and finally eluted with 0.3 M methyl α-mannopyranoside, 0.1 mM $CaCl_2$ (arrow) in 0.1 M PB, pH 7.2; flow: 0.5 ml min^{-1}. Five ml fractions were collected and assayed for benzylamine oxidase activity. (c) Chromatography of 1 ml amine oxidase from ConA sepharose on a mono Q column. After application of the sample, the column was eluted with a linear gradient from 50 mM PB, pH 7.2 to 50 mM PB, pH 7.2, 1 M NaCl over 60 min; flow: 0.5 ml min^{-1}. Half-millilitre fractions were collected and assayed for benzylamine oxidase activity.

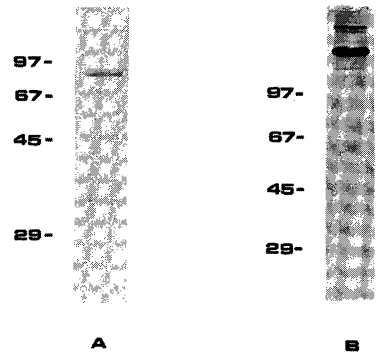


Fig. 2. SDS-PAGE analysis of purified bovine serum amine oxidase. Samples were boiled in electrophoresis sample buffer (1 : 1) with 40 mM DTT (A) or without DTT (B) and analysed on 11% (A) or 10% (B) gels.

human placental diamine oxidase and human pregnancy serum diamine oxidase were purified. All the enzymes showed monomer and dimer M_rs of 90 kDa and 180 kDa, respectively (not shown).

The purified enzymes were subjected to amino acid analysis and peptide mapping studies, in order to assess the relationship between the enzymes and to obtain amino acid sequence information.

All of the enzymes were resistant to cleavage with trypsin as also described recently by Janes *et al.*[9] and released only a few hydrophilic peptides. However, similar profiles were seen in HPLC chromatograms of the limited trypsin digests (Fig. 3). BSAO was the enzyme most susceptible to trypsin and V8 treatment and could be digested more extensively in the presence of 20% organic solvent resulting in the profiles shown in Fig. 10. Selected peaks from the chromatograms were subjected to automated Edman sequencing whereby the sequences in Table 1 were

Table 1. Amino acid sequences of peptides derived from BSAO.[a]

Peptide	Enzyme	Sequence
1	T	AFsLG3
2	T	ALDPADWTVQK
3	T	STWFGIYYxITK
4	T	E*GQDAGSQE*INPLAQL
5	T	NQNDPWTPTVDFSDFINxETIAGK
6	T	VDLDVGGLE*NwVwAE*D*MAFVPTAIP*
7	V8	xQxAGSxE
8	V8	NWVWAE
9	V8	MAFVPTAIpNSe

[a] Peptides from HPLC were applied directly to the Polybrene-coated glass filter and subjected to automated Edman degradation. Sequences in the tryptic peptides which were also found in the *S. aureus* V8 peptides are italicized. Capital letters indicate that the residue was unambiguously identified. Lower-case letters indicate that the residue was identified with a high degree of certainty. x denotes positions where no residue was identified.

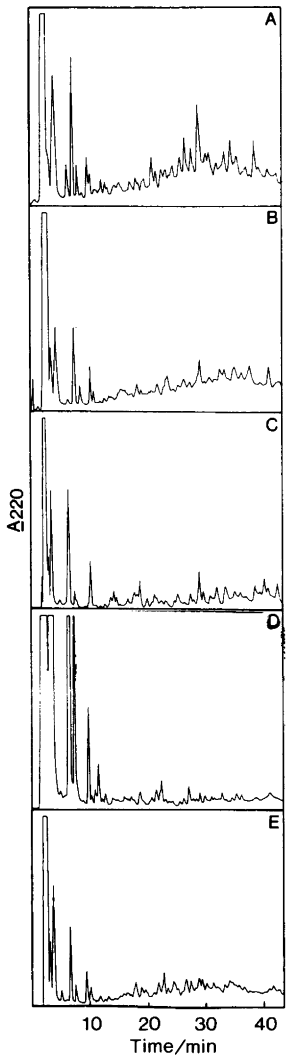


Fig. 3. HPLC profiles of limited tryptic digests of purified amine oxidases: A, BSAO; B, PKDAO; C, HPSDAO; D, HPDAO 1; E, HPDAO 2. The samples (100 µl, 1 mg ml^{-1}) were incubated with trypsin 1 : 1000 (w : w) at 37°C overnight. The reaction was stopped by addition of 10 µl 10% TFA. Hundred µl aliquots were analysed on a C-18 column (2.1 mm × 220 mm) eluted with a gradient of CH_3CN in 0.1% TFA.

Table 2. Cross-reactivity table for monoclonal antibodies against amine oxidases tested against purified amine oxidases by dot blot and Western blot.[a]

	Enzyme			
Antibody	BSAO	HPSDAO	HPDAO	PKDAO
1 BSAO 10 D9-4	+++	+	+	++
2 BSAO 7C6-2	+++	+	+	++
3 BSAO 10E5-3	+++	++	+	++
4 HPDAO 40-6	+	+	+++	+/−
5 HPDAO 40-2	++	+++	+++	+/−
6 HPSDAO 869-3	+	+++	+	+/−
7 Control mab 8DY	−	−	−	−

[a] +++, strong reactivity; ++, medium reactivity; +, weak reactivity; −, no reactivity.

obtained. The enzymes were susceptible to degradation with pepsin (Fig. 4) but the resulting peaks isolated from the HPLC were, in most cases, found to contain several coeluting peptides, and were not suitable for direct sequence analysis.

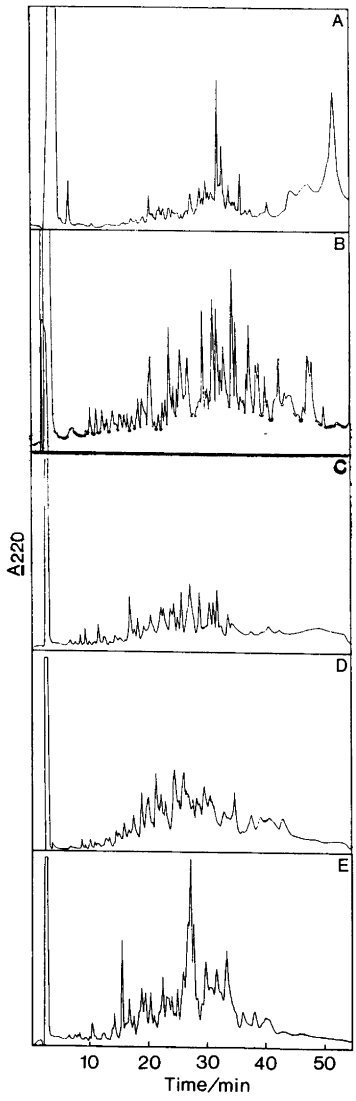


Fig. 4. HPLC profiles of various proteolytic digests of purified amine oxidases: A BSAO (300 µg in 300 µl PB pH 7.2 with 10% MeOH) digested with *S. aureus* V8 protease (1 : 100 w : w) in the presence of organic solvent (10% MeOH). The reaction was carried out overnight at room temp. and was terminated by addition of 10 µl 10% TFA. Hundred µl were analysed on a 0.5 cm × 10 cm RP-18 column (Waters, Novapak). B, BSAO digested extensively with trypsin. A hundred µg in 100 µl PB pH 7.2 were treated with trypsin (1 : 10 w : w) at room temp. for 10 h. The reaction was stopped by addition of 10 µl 10% TFA. A hundred µl of the reaction mixture were analysed on a 2.1 mm × 220 mm RP18 column. C–E, digestions of BSAO (C), HPSDAO (D) and HPDAO(E) with pepsin analysed by HPLC as in B. Samples were incubated with pepsin (1 : 100, w : w) for 1 h at 37°C. Reactions were stopped by addition of 10% TFA and the reaction mixture was injected into the HPLC.

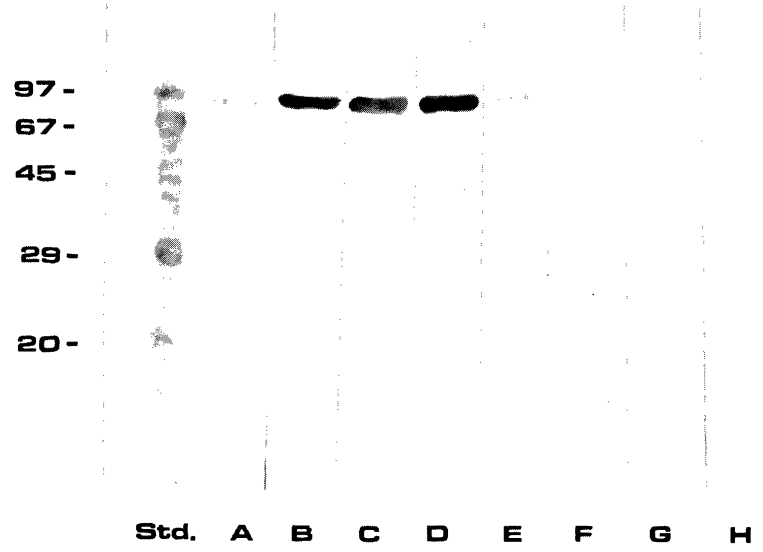


Fig. 5. Western blots using BSAO purified by monoclonal antibody 10 D9-4 and incubated with monoclonal antibodies: A, coomassie stained strip; B, 10 D9-4; C, 7C6-2; D, 10E5-3; E–F, anti-HPDAO; E, Hyb 40-6; F, Hyb 40-2; G–H, *anti* HPSDAO; G, HPSDAO 869-3; H, HPSDAO 8DY.

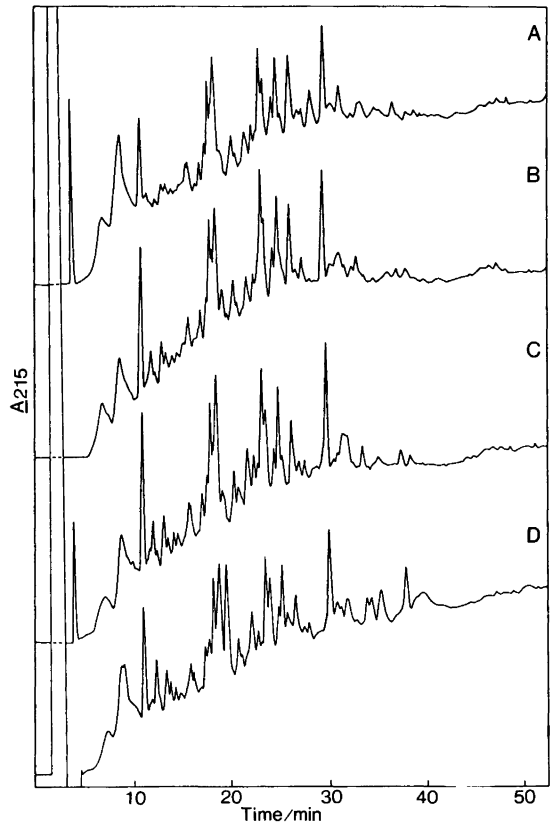


Fig. 6. Peptide maps of immunopurified BSAO (A), PKDAO (B), HPSDAO (C), HPDAO (D). Individual peaks from the chromatograms were collected for subsequent sequence analysis.

The purified bovine serum amine oxidase, human placental and human pregnancy serum diamine oxidases were used for raising monoclonal antibodies, which were in turn used for purifying amine oxidases from the various sources. The immunopurified enzymes were used for studying cross-reactivity between the various monoclonal antibodies. In Fig. 5 are shown results of Western blots using bovine serum amine oxidase purified by one monoclonal antibody (10 D9-4) and subsequently incubated with monoclonal antibodies to BSAO, HPDAO and HPSDAO. As seen the monoclonal antibodies to BSAO all cross-reacted strongly with the BSAO purified by monoclonal antibody 10 D9-4 whereas monoclonal antibodies to HPDAO cross reacted more weakly and one monoclonal antibody to HPSDAO cross-reacted with BSAO while one monoclonal antibody to HPSDAO did not show cross reactivity. Extending these studies to amine oxidases purified by the monoclonal antibodies from the four different sources and using both Western blots and dot blots the cross reactivity results shown in Table 2 were obtained. Generally, all the antibodies cross-react with all four enzymes, but the strongest cross-reactivity is seen between BSAO and PKDAO and between HPDAO, BSAO and HPSDAO. Peptide mapping studies using the immunopurified enzymes are shown in Fig. 6. In these studies the enzymes were digested with trypsin in the presence of 20% MeOH since the addition of organic solvent had previously been found to improve the digestion of the bovine serum amine oxidase. Although relatively few peptides were released by trypsin digestion, it can be concluded that the peptide maps are very similar.

Discussion

Cu-containing amine oxidases have previously been purified and characterized from porcine and human kidney, human placenta, porcine and bovine serum and human pregnancy serum.[1,7–10,12,20,22–24] These studies showed that the Cu-containing amine oxidases have similar compositions with a monomer M_r of 90 kDa, and a dimer M_r of 180 kDa and form multimers of higher M_r. The Cu content is one Cu atom per monomer and the content of organic prosthetic group is a minimum of one per dimer. The enzymes contain about 10% carbohydrate and have isoelectric points between 4.5 and 6.5. In these studies, where the enzymes from different tissues and body fluids within the same species were compared, the enzymes were found to be identical with respect to the properties compared, i.e., substrate specificites, M_r, immunological properties, and structure. A comparison of published values for amino acid composition of PKDAO, HPSDAO, PSAO and BSAO showed a close relationship between the enzymes (not shown), in line with the above results. In the present study, the four amine oxidases could be purified by ammonium sulfate precipitation, AH-Sepharose chromatography, ConA Sepharose chromatography and ion-exchange chromatography. The purified proteins showed bands of M_r 90000 and 180000 upon SDS-PAGE, corresponding to the enzyme monomer and dimer, respectively.

The four enzymes, although resistant to proteolytic digestion, showed similar peptide patterns in HPLC chromatograms of tryptic digests, although differences in the peptide patterns were also found. The enzymes were susceptible to pepsin digestion. The bovine enzyme could be more extensively digested by trypsin and *S. aureus* V8 enzyme in the presence of organic solvent and several peptides were isolated and sequenced. The sequences obtained showed no homology to known sequences. The purified enzymes were also used for obtaining mouse monoclonal antibodies. Most of the antibodies showed cross-reactivity with the different purified enzymes. Together these results indicate that the Cu-amine oxidases form a closely related group of enzymes (E.C. 1.4.3.6). Determination of the exact number of enzymes, however, must await further studies, but it seems clear that a close relationship exists between the enzymes, with differences in structure reflecting the differences in substrate specificity.[1]

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