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SOLUBILIZATION AND PURIFICATION OF A-ESTERASE FROM MOUSE HEPATIC MICROSOMES

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Abstract—A-esterase(s), an enzyme(s) that hydrolyzes certain organophosphate compounds, is located in mammals, primarily in serum and liver. Although considerable information is available regarding serum A-esterase(s), little is known about the hepatic form(s) of this enzyme. In the present study, hepatic A-esterase activity was quantified by measuring the EDTA-sensitive hydrolysis of the organophosphate paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphate). EDTA-insensitive hydrolysis was assumed to be the nonenzymatic phosphorylation of proteins with appropriate serine hydroxyl groups. Resuspension of mouse hepatic microsomes in 50 mM potassium phosphate buffer, pH 7.4, containing 100 μM calcium chloride, 0.25% sodium cholate, and 0.1% Triton N-101, resulted in the solubilization of A-esterase activity, as evidenced by the failure of activity to sediment after centrifugation at 100,000 *g* for 1 hr. Gel permeation chromatography followed by ion-exchange chromatography and nonspecific affinity chromatography resulted in a peak of A-esterase activity judged to be homogeneous by SDS–PAGE. A typical purification resulted in a 1531-fold increase in specific activity, with a recovery of 10%. SDS–PAGE with and without an acrylamide gradient indicated a molecular weight of 40,000 and 39,000 Da, respectively, while analyses of amino acid composition revealed similarities with human and rabbit serum paraoxonase. And finally, although this protein hydrolyzed both paraoxon and methyl paraoxon (*O*, *O*-dimethyl-*O*-*p*-nitrophenyl phosphate), it did not hydrolyze *p*-nitrophenyl acetate.

Key words: A-esterase; paraoxonase; arylesterase; organophosphate; parathion; paraoxon

Phosphorothioate insecticides, such as parathion (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate) and chlorpyrifos (*O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate), are commonly used throughout the world. These chemicals have little capacity to inhibit acetylcholinesterase themselves, but instead are metabolically activated by cytochromes P450 to form potent acetylcholinesterase inhibitors termed oxons or oxygen analogs [1, 2]. Since these oxons are extremely toxic, those factors that control their pharmacokinetic disposition can greatly affect the degree of toxicity observed following exposure to phosphorothioate insecticides. The mammalian detoxification of many oxygen analogs, like paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphate) and chlorpyrifos oxon (*O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphate), is catalyzed by an enzyme(s) that has been referred to by many different names, including aryl-ester hydrolase (EC 3.1.1.2), arylesterase, A-esterase, paraoxonase, organophosphate hydrolase, and aryldialkyl phosphatase (EC 3.1.8.1) (Fig. 1) [3–7]. More recently, the terms organophosphorus acid anhydrolase and organophosphorus compound hydrolase have also been suggested [5, 8]. Much of the confusion regarding nomenclature has resulted from the conflicting evidence describing those substrates hydrolyzed by this enzyme(s) [6–9]. Furthermore, terms like paraoxonase can apply to enzymes that are clearly different, but that possess the capacity to hydrolyze paraoxon. For example, while a paraoxonase has been purified from *Pseudomonas diminuta* [10], this enzyme, which requires zinc and can also hydrolyze parathion [10, 11], is clearly different from mammalian paraoxonase, which cannot hydrolyze parathion and requires calcium for activity [7]. Moreover,

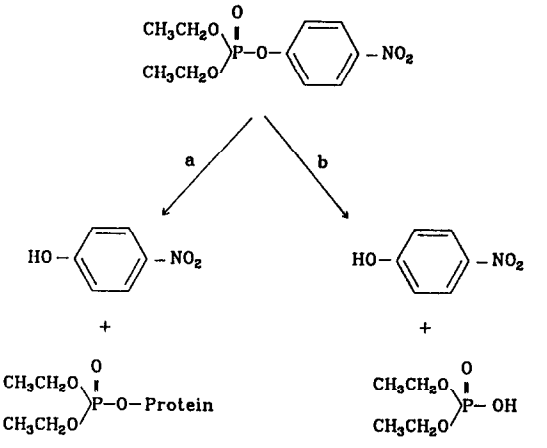

Fig. 1. Mammalian biotransformation of paraoxon. Reaction *b* is catalyzed by A-esterase(s), while reaction *a* represents nonenzymatic phosphorylation of proteins with appropriate hydroxyl groups.

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mammalian organophosphate hydrolytic activity can be divided into two categories: the first hydrolyzes DFP* and requires cobalt, magnesium, or manganese for activity, whereas the second hydrolyzes paraoxon and requires calcium for activity [7]. In the present study, the term A-esterase is used to refer to an enzyme(s) that hydrolyzes the organophosphate paraoxon, and is inhibited by EDTA.

A-esterase has been identified in several mammalian tissues, with the highest activity in blood and liver [9, 12–14]. Yet, at present, this enzyme(s) has no known function *in vivo*, although serum A-esterase has been reported to be associated with HDLs [15–17]. Both serum and hepatic A-esterase require calcium and a free sulfhydryl(s) for activity [18, 19]. In addition, human serum A-esterase has been reported to be polymorphic in nature, existing as high-activity and low-activity forms [20–23]. Recently, human and rabbit serum A-esterase has been purified and the cDNA characterized [6, 24, 25]. However, compared with serum A-esterase, little is known about hepatic A-esterase, perhaps due to its association with the endoplasmic reticulum. Therefore, the present study, utilizing paraoxon as a substrate, was directed towards the solubilization and purification of A-esterase from mouse liver.

MATERIALS AND METHODS

Chemicals

Paraoxon and methyl paraoxon (*O,O*-dimethyl-*O*-*p*-nitrophenyl phosphate) were synthesized as previously described [26–28]. The reader is reminded that both of these chemicals are highly toxic, and special safety precautions must be observed. Sephacryl S-300 and DEAE-Sepharose were purchased from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ); all electrophoresis reagents were supplied by Bio-Rad Laboratories (Richmond, CA). Sodium cholate, Triton N-101, Cibacron Blue 3GA, and *p*-nitrophenyl acetate were purchased from the Sigma Chemical Co. (St Louis, MO).

Animals

Male Swiss Webster mice (25–35 g body weight), obtained from Taconic Farms (Germantown, PA), were used in all experiments except those evaluating sex differences, which utilized females and males, and were housed under standard laboratory conditions with free access to water and feed (Purina Rodent Chow).

Analytical procedures

Isolation and solubilization of microsomes. All procedures were done at 0–4°, unless otherwise indicated. Following decapitation of mice, their livers were removed, homogenized in 3 vol. of 50 mM potassium phosphate buffer (pH 7.4) containing 100 μM calcium chloride (buffer 1). In certain cases, samples were frozen at −70° for up to 1 month. Homogenates were centrifuged at 10,000 *g* for 20 min. After careful aspiration of the fatty layer, the supernatants were removed and centrifuged at 100,000 *g* for 1 hr. The microsomal pellets were resuspended in 3 vol. of 50 mM potassium phosphate buffer, pH 7.4, containing 100 μM calcium chloride, 0.25% sodium cholate, and 0.1% Triton N-101 (buffer 2). Following gentle stirring for 30 min, the sample was centrifuged at 100,000 *g* for 1 hr. The volume of the supernatant was reduced by ultrafiltration (using a membrane with a molecular weight cutoff of 10,000) to 20% of its original volume.

Gel permeation chromatography. Sephacryl S-300 was resuspended and washed in buffer 2, and packed in a 2.5 × 100 cm column (column bed: 2.5 × 83 cm). Solubilized supernatant was loaded onto the column in a volume not greater than 15 mL, and was eluted by ascending flow with buffer 2 at a flow rate of 0.75 mL/min. Fractions of 3 mL/tube were collected. Those fractions containing peak A-esterase activity (present in an elution volume of about 236–256 mL) were pooled.

Ion-exchange chromatography. A DEAE-Sepharose column (column bed: 1.5 × 40 cm) was equilibrated with buffer 1. After loading the sample (about 12 mL), a linear, 200 mL sodium chloride gradient (0–500 mM) was begun. The flow rate was maintained at 0.5 mL/min, and fractions of 3 mL were collected. A single peak of A-esterase activity (peak A) was identified in the column eluate, ranging from about 132 to 152 mL elution volume. The column was subsequently washed with 100 mL of buffer 1. A linear 200 mL sodium chloride gradient (0–300 mM) in buffer 2 was begun. A peak of A-esterase activity (peak B) eluted in 460–480 mL.

Nonspecific affinity chromatography. A Cibacron Blue 3GA column (column bed 2.0 × 15 cm) was washed and equilibrated in 50 mM Tris–HCl buffer, pH 8.0, with 100 μM calcium chloride (buffer 3), containing 3 M sodium chloride. This chromatographic step was performed at room temperature. Two volumes of buffer 3 containing 3 M sodium chloride were added to peak B from the DEAE-Sepharose eluate, and the sample was loaded onto the Cibacron Blue 3GA column. The column was washed with buffer 3 until protein could not be detected in the eluate (about 200 mL). A 200 mL linear gradient of 0–0.2% sodium cholate in buffer 3 was applied to the column at a flow rate of 0.8 mL/min. Fractions of 3 mL were collected.

Analytical methods. To locate quickly those fractions of column eluates that contained A-esterase, 0.5 mL of each fraction was added to a series of tubes containing 1.5 mL of buffer 1, as well as to a series of tubes with identical contents, but also containing 6.7 mM EDTA. After addition of 1000 nmol paraoxon to each sample, and incubation at 37° for 30 min, the absorbance at 398 nm was monitored on a Stazar spectrophotometer (Gilford, Oberlin, OH). An extinction coefficient for *p*-nitrophenol of 10,600 M^{-1} cm^{-1} was used. Since enzymatic hydrolysis of paraoxon by A-esterase requires calcium, total activity minus EDTA-insensitive activity yielded A-esterase activity. EDTA-insensitive hydrolysis was assumed to result

* Abbreviations: DFP, diisopropylflurophosphate; HDL, high-density lipoprotein; cDNA, complementary DNA.

from nonenzymatic phosphorylation of serine hydroxyl groups [29].

For more accurate determinations of A-esterase specific activity (such as those used for pooled samples of column eluates), a Shimadzu MPS-2000 UV–vis spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) was used. The reference cuvette contained 0.25 mL sample and 2.25 mL of buffer 1; the sample cuvette contents were identical, except for the addition of 1000 nmol of paraoxon in 8.3 μL of ethanol. A second assay was performed under identical conditions except that 6.7 mM EDTA was present within both sample and reference cuvettes. Total activity minus EDTA-insensitive activity gave A-esterase. In certain instances, reactions were carried out in semi-micro cuvettes in order to conserve sample. In these instances the cuvette contents were reduced by one-half. Protein content was determined by the Coomassie Protein Assay Reagent (Pierce Chemical Co., Rockford, IL) [30].

Gel electrophoresis was performed as described by Laemmli [31]. Slab gels, containing 10% acrylamide in the presence of 0.1% SDS, were used at room temperature. Stacking gels contained 5% acrylamide. Prior to loading, samples were mixed with an equal volume of 62.5 mM Tris–HCl, pH 6.8, containing 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.1% bromophenyl blue, and heated at 100° for 5 min. The samples were run on constant current at 20 mA for 1 hr, followed by 30 mA for 5 hr. Protein was stained with 0.2% Coomassie Blue G250 in 7% acetic acid for 6 hr, and destained with 7% acetic acid. Gels were scanned, and molecular weights were determined on a GS-300 scanning densitometer with the GS-365 Data System (Hoefer Scientific Instruments, San Francisco, CA). Gradient SDS–PAGE was performed as just described, except that a 5–30% acrylamide gradient was used. In addition, the gel was run on constant voltage at 100 V for 16 hr [32].

Table 1. EDTA-sensitive hydrolysis of paraoxon by cytosol or microsomes of perfused and non-perfused livers from male and female mice

Sex	Subcellular fraction	*p*-Nitrophenol formation* (nmol/mg protein/min) Perfused livers†	Nonperfused livers†
Male	Cytosol	ND‡	0.29 ± 0.10
Female	Cytosol	ND	0.19 ± 0.09
Male	Microsomes	2.21 ± 0.28	2.14 ± 0.35
Female	Microsomes	2.01 ± 0.40	2.09 ± 0.35

* Each value represents the mean ± SD of four mice.

† Following ether anesthesia, mice had their livers perfused through the hepatic portal vein with 50 mM sodium phosphate buffer (pH 7.4) containing 4% BSA, for 3 min in order to remove residual blood.

‡ No activity detected.

Amino acid composition was provided by the Rockefeller University Sequencing Facility (Rockefeller University, New York, NY), which is supported in part by NIH shared instrumentation grants and by funds provided by the U.S. Army and Navy for purchase of equipment. Samples were prepared for analyses according to directions provided by the Rockefeller University Sequencing Facility.

RESULTS

A-esterase activity detected in mouse hepatic cytosol was eliminated by perfusion of livers *in situ* (Table 1), suggesting that activity within the cytosol was contamination from residual blood after decapitation. Additionally, no sex-related differences were observed in microsomal A-esterase activities (Table 1). Maximal solubilization of microsomal A-esterase activity, as evidenced by non-sedimentation

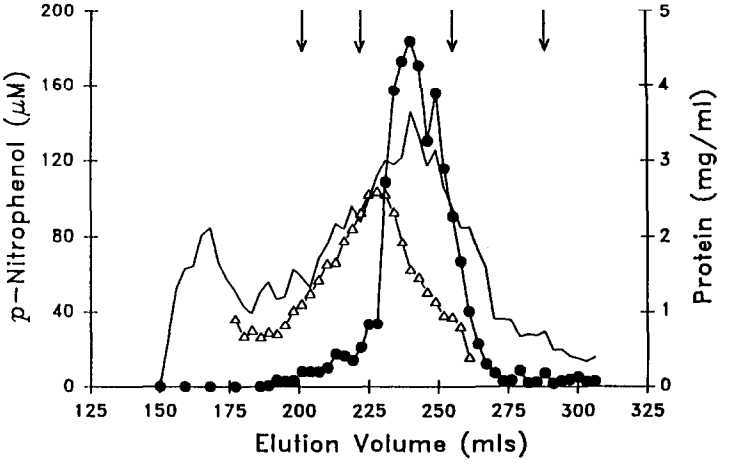

Fig. 2. Sephacryl S-300 chromatography of solubilized mouse liver microsomes. The closed circles and open triangles represent *p*-nitrophenol production that was sensitive and insensitive, respectively, to EDTA, while the solid line represents total protein. The elution volumes for standards are indicated by the arrows. Standards were, from left to right, as follows: thyroglobulin (669,000), ferritin (440,000), catalase (232,000), and aldolase (158,000).

Table 2. Purification of A-esterase

Fraction	Volume (mL)	Total protein (mg)	Specific activity*	Purification (fold)	Recovery (%)
Solubilized microsomes	280	476.6	0.28	1.0	100
Sephacryl S-300 eluate	53	99.1	1.26	4.5	95
DEAE-Sepharose eluate	18	1.4	50.89	81.8	54
Cibacron Blue eluate	16	0.03	428.57	1530.6	10

* Expressed as nmol *p*-nitrophenol formed/mg protein/min.

of activity at 100,000 *g* for 1 hr, was achieved by resuspension of microsomes in 3 vol. of buffer containing 0.25% sodium cholate and 0.1% Triton N-101. Under these conditions, 56% of the total microsomal protein was recovered in solubilized supernatant, with the remainder in the unsolubilized pellet. However, following addition of the detergents to microsomes, the specific activity of A-esterase dropped immediately to about one-third of that of unsolubilized microsomes (data not shown). Alterations in detergent concentrations (including various concentrations of Triton N-101 in the absence of sodium cholate) resulted in less solubilized activity than did 0.25% sodium cholate and 0.1% Triton N-101 (data not shown).

Gel filtration of solubilized microsomes on Sephacryl S-300 resulted in a peak of A-esterase activity that eluted with an apparent Stoke's radius of 57.7, corresponding to a molecular mass of 330,522 Da (Fig. 2). As a result of the gel filtration step, A-esterase activity was enriched about 5-fold (Table 2). A substantial amount of EDTA-insensitive paraoxon-hydrolyzing activity was also identified in effluent from the Sephacryl S-300 column (Fig. 2). This activity likely resulted from the presence of certain proteins containing serine hydroxyl groups, which can be phosphorylated by paraoxon [29].

Although ion-exchange chromatography revealed two peaks of A-esterase activity (Fig. 3), the present study was directed towards the characterization of the second peak eluted (peak B, Fig. 3). Final purification of peak B from the ion-exchange column was achieved through use of Cibacron Blue 3GA, first utilized by Gan *et al.* [6]. As was the case with the ion-exchange step, two peaks were apparent in the eluate of the Cibacron Blue 3GA column (Fig. 4). However, the present study characterized the larger of the two peaks. A typical purification resulting in 1531-fold purification and a recovery of 10% is presented in Table 2. SDS–PAGE with and without an acrylamide gradient indicated the presence of one major protein band with a molecular weight of 40,000 and 39,000 Da, respectively (Figs. 5 and 6). Previously reported molecular weights for human and rabbit serum A-esterase are 43,000 [6] and 35,000/38,000 Da [24], respectively. The amino acid composition of mouse hepatic A-esterase is presented in Table 3.

Methyl paraoxon, an organophosphate similar in structure to paraoxon, was much less avidly hydrolyzed by A-esterase, compared with paraoxon (final specific activities of 70.71 and 428.47 nmol/mg protein/min, respectively). In addition, A-esterase did not hydrolyze *p*-nitrophenyl acetate (data not shown).

DISCUSSION

The reduction of hepatic microsomal A-esterase activity after addition of sodium cholate and Triton

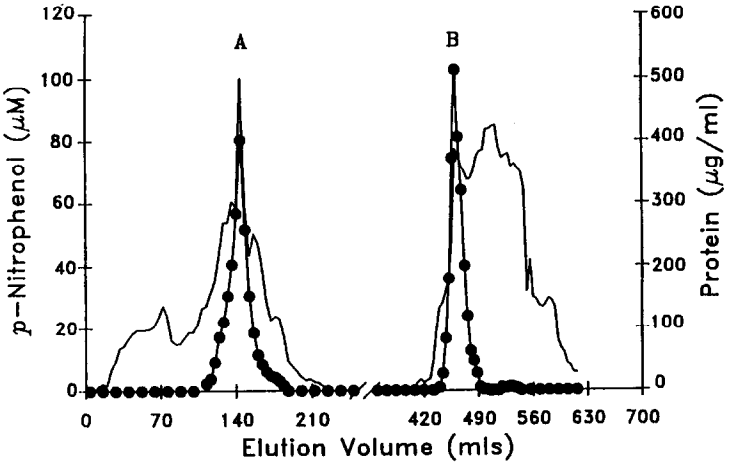

Fig. 3. Chromatographic profile of A-esterase activity on DEAE-Sepharose. The closed circles represent A-esterase activity, while the solid line represents total protein. Two peaks of A-esterase (Peak A and Peak B) were identified.

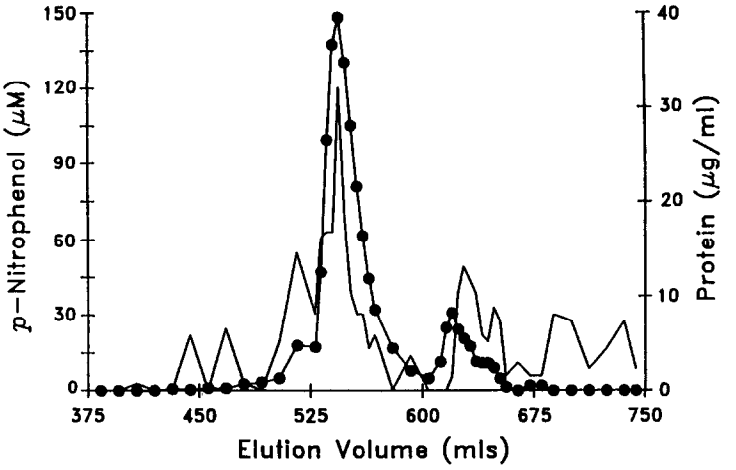

Fig. 4. Chromatographic profile of A-esterase activity on Cibacron Blue 3GA. The closed circles represent A-esterase activity, while the solid line represents total protein.

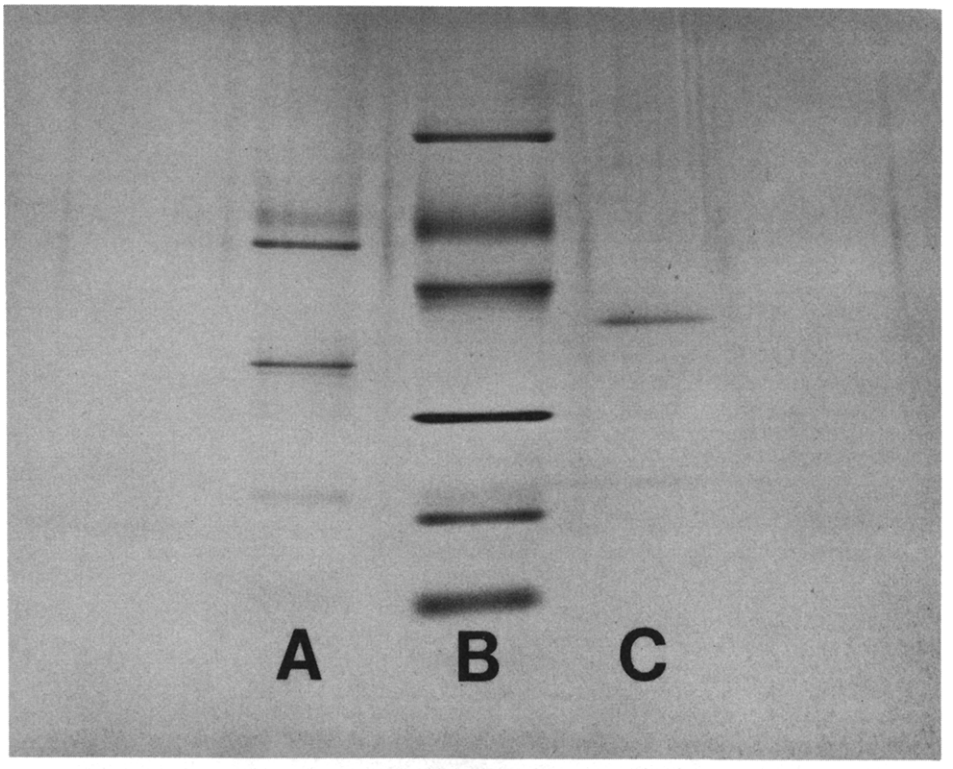

Fig. 5. Gradient SDS–PAGE of purified mouse hepatic microsomal A-esterase. Lane A contained the standards catalase (60,000) and lactate dehydrogenase (36,000). Lane B contained phosphorylase *b* (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400). Lane C contained 10 μg of the Cibacron Blue eluate containing A-esterase activity.

N-101 could have resulted from direct inhibition of enzyme activity by detergent, as occurs with enzymes like cytochromes P450 at high detergent concentrations [33], or from conformational changes in the protein upon solubilization. Recovery of activity could not be improved by alterations in detergent concentrations, or by addition of 20% glycerol and/or 10 mM dithiothreitol to the solubilization buffer (data not shown).

Both the ion-exchange chromatography and the nonspecific affinity chromatography yielded two peaks of hydrolytic activity towards paraoxon, which were inhibited by EDTA (Figs 3 and 4). While these could represent multiple forms of A-esterase,

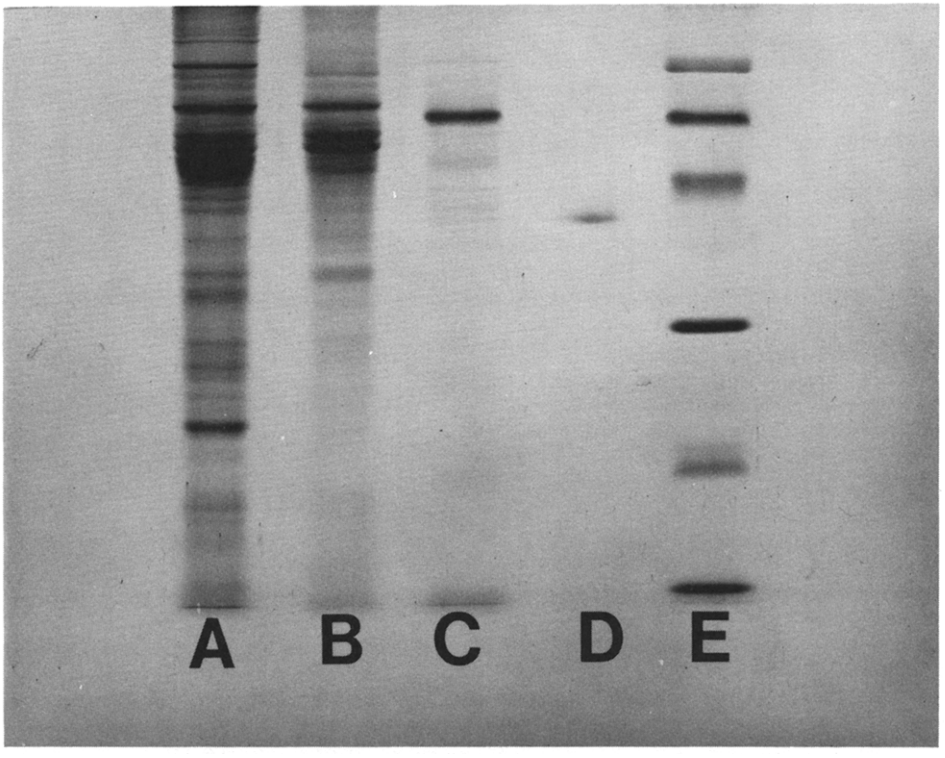

Fig. 6. SDS–PAGE of purified mouse hepatic microsomal A-esterase. Lane A contained 20 μg of solubilized microsomes. Lane B contained a 20 μg sample of the Sephacryl S-300 eluate. Lane C contained 10 μg of the DEAE-Sepharose eluate. Lane D contained 10 μg of the Cibacron Blue eluate. Lane E contained the following standards: phosphorylase *b* (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400).

Table 3. Amino acid composition (molar %) of mouse hepatic, rabbit serum, and human serum A-esterase*

Amino acid	Mouse	Human	Rabbit
Ala	3.4	3.2	3.5
Arg	2.2	2.4	2.3
Asx	15.7	11.5	12.6
Cys	0.0	0.8	0.8
Glx	9.6	10.9	8.0
Gly	0.0	3.4	3.3
His	1.8	4.1	4.4
Ile	9.9	5.4	5.4
Leu	19.8	12.8	11.8
Lys	9.4	6.4	7.7
Met	2.6	2.3	2.3
Phe	6.2	6.5	6.6
Pro	0.0	4.6	5.1
Ser	3.4	5.2	5.2
Thr	1.2	4.8	4.8
Trp	0.0	2.0	2.0
Tyr	4.9	7.0	6.5
Val	10.1	6.7	7.7

* The human and rabbit compositions were calculated from data presented by Hassett *et al.* [25].

the possibility also exists that they are the same protein which has undergone modification(s) during purification. However, it should be noted that based on kinetic analyses of the biotransformation of paraoxon and chlorpyrifos oxon, Sultatos and Murphy [19] concluded that more than one microsomal A-esterase existed in the mouse. Similarly, the existence of several forms of A-esterase associated with the HDL fraction in sheep serum has been reported previously [34]. Moreover, Haas and Geldmacher-von Mallinckrodt [35], utilizing isoelectric focusing, documented the presence of multiple bands in human serum which displayed EDTA-sensitive hydrolysis of paraoxon. While Gan *et al.* [6] purified human serum A-esterase to give a single band on SDS–PAGE with a molecular weight of 43,000 Da, Furlong *et al.* [24] reported two bands of purified rabbit A-esterase with molecular weights of 35,000 and 38,000 Da, and two bands of purified human A-esterase with molecular weights of 44,700 and 47,900 Da. They speculated that the two bands could have resulted from differences in carbohydrate content [24].

The amino acid composition of mouse hepatic A-esterase is similar to that of paraoxonase from human and rabbit serum previously reported by Hassett *et al.* [25] (Table 3). However, the absence of Cys, Gly, Pro, or Trp in mouse hepatic A-esterase must be interpreted cautiously, particularly since a free sulfhydryl group(s) has been reported to be required for activity [19]. Consequently, the failure to detect these amino acids could reflect their low levels in this protein (rather than their complete absence), as is the case with human and rabbit serum paraoxonase

(Table 3). It should be pointed out that the amino acid compositions of human and rabbit serum paraoxonase in Table 3 were not determined directly, but were calculated from the amino acid sequences deduced from the cDNA reported by Hassett *et al.* [25].

Previous reports concerning the substrate specificity of A-esterase have been inconsistent. Although the original definition of A-esterase put forth by Aldridge [36] referred to those esterases that were insensitive to inhibition by organophosphorus compounds and hydrolyzed carboxylic esters, Aldridge [12] subsequently demonstrated an association between this activity and the hydrolysis of paraoxon. While evidence exists indicating that human A-esterase (paraoxonase) is both an aryldialkyl-phosphatase (EC 3.1.8.1) as well as an arylesterase (EC 3.1.1.2) [6, 24], some reports claim they are entirely separate enzymes [7, 8]. In the present study, mouse hepatic microsomal A-esterase hydrolyzed both paraoxon and methyl paraoxon, but not *p*-nitrophenyl acetate (data not shown). However, since A-esterase had to be solubilized in order to be purified, the substrate specificity of this enzyme could have been altered by the detergents and/or the removal of the lipid environment. Therefore these data cannot discount the hypothesis that A-esterase is both an aryldialkyl-phosphatase as well as an arylesterase.

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REFERENCES

1. Eto M, *Organophosphorus Pesticides: Organic and Biological Chemistry*. CRC Press, Boca Raton, FL, 1979.
2. Forsyth CS and Chambers JE, Activation and degradation of the phosphorothionate insecticides parathion and EPN by rat brain. *Biochem Pharmacol* **38**: 1597–1603, 1989.
3. Aldridge WN, A-esterases and B-esterases in perspective. In: *Enzymes Hydrolyzing Organophosphorus Compounds* (Eds. Reiner E, Aldridge WN and Hoskin FCG), pp. 1–14. Ellis Horwood Limited, West Sussex, England, 1989.
4. Heymann E, A proposal to overcome some general problems of the nomenclature of esterases. In: *Enzymes Hydrolyzing Organophosphorus Compounds* (Eds. Reiner E, Aldridge WN and Hoskin FCG), pp. 226–235. Ellis Horwood Limited, West Sussex, England, 1989.
5. Aldridge WN, Hoskin FCG, Reiner E and Walker CH, Suggestions for a nomenclature and classification of enzymes hydrolyzing organophosphorus compounds. In: *Enzymes Hydrolyzing Organophosphorus Compounds* (Eds. Reiner E, Aldridge WN and Hoskin FCG), pp. 246–253. Ellis Horwood Limited, West Sussex, England, 1989.
6. Gan KN, Smolen A, Eckerson HW and La Du B, Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metab Dispos* **19**: 100–106, 1991.
7. Mackness MI, 'A'-esterases. Enzymes looking for a role? *Biochem Pharmacol* **38**: 385–390, 1989.
8. Hoskin FCG, Steeves DM, Walker JE and Woodbury CT, Polyclonal antibody generation in rabbit by administration of an organophosphorus acid anhydrolase (OPAA) from squid. *Biochem Pharmacol* **46**: 1223–1227, 1993.
9. Mackness MI, Thompson HM, Hardy AR and Walker CH, Distinction between 'A'-esterase and arylesterases. *Biochem J* **245**: 293–296, 1987.
10. Dumas DP, Caldwell SR, Wild JR and Raushel FM, Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. *J Biol Chem* **264**: 19659–19665, 1989.
11. Omburo GA, Kuo JM, Mullins LS and Raushel FM, Characterization of the zinc binding site of bacterial phosphotriesterase. *J Biol Chem* **267**: 13278–13283, 1992.
12. Aldridge WN, Serum esterases 2. An enzyme hydrolyzing diethyl *p*-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochem J* **53**: 117–124, 1953.
13. Chemnitius J-M, Losch H, Losch K and Zech R, Organophosphate detoxicating hydrolases in different vertebrate species. *Comp Biochem Physiol* **76C**: 85–93, 1983.
14. Shishdo T and Fukami JI, Enzymatic hydrolysis of diazoxon by rat tissue homogenate. *Pestic Biochem Physiol* **2**: 39–50, 1972.
15. Mackness MI and Walker CH, Partial purification and properties of sheep serum 'A'-esterases. *Biochem Pharmacol* **32**: 2291–2296, 1983.
16. Mackness MI, Hallam SD, Peard T, Warner S and Walker CH, The separation of sheep and human serum A-esterase activity into the lipoprotein fraction by ultracentrifugation. *Comp Biochem Physiol* **82B**: 675–677, 1985.
17. Mackness MI, Hallam SD and Walker CH, 'A'-esterase activity in the lipoprotein fraction of sheep and human serum. *Biochem Soc Trans* **13**: 135–136, 1985.
18. Junge W and Krisch K, Current problems on the structure and classification of mammalian liver carboxylesterases (EC 3.1.1.1). *Mol Cell Biochem* **1**: 41–52, 1973.
19. Sultatos LG and Murphy SD, Hepatic microsomal detoxification of the organophosphates paraoxon and chlorpyrifos oxon in the mouse. *Drug Metab Dispos* **11**: 232–238, 1983.
20. Krisch K, Enzymatische hydrolyse von diathyl-*p*-nitrophenylphosphat (E600) durch menschliches serum. *Z Klin Chem Klin Biochem* **1**: 41–45, 1968.
21. Playfer JR, Eze LC, Bullen MF and Evans DAP, Genetic polymorphism and interethnic variability of plasma paraoxonase activity. *J Med Genet* **13**: 337–342, 1976.
22. Carro-Ciampi G, Gray S and Kalow W, Paraoxonase phenotype distribution in Canadian Indian and Inuit populations. *Can J Physiol Pharmacol* **61**: 336–340, 1983.
23. Eckerson HW, Ramson J, Wyte C and La Du BN, The human paraoxonase polymorphism: Identification of phenotypes by their response to salts. *Am J Hum Genet* **35**: 214–227, 1983.
24. Furlong CE, Richter RJ, Chapline C and Crabb JW, Purification of rabbit and human serum paraoxonase. *Biochemistry* **30**: 10133–10140, 1991.
25. Hassett C, Richter RJ, Humbert R, Chapline C, Crabb JW, Omiecinski CJ and Furlong CE, Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. *Biochemistry* **30**: 10141–10149, 1991.
26. Sultatos LG, Basker KM and Murphy SD, The interaction of the phosphorothioate insecticides chlorpyrifos and parathion, and their oxons with bovine serum albumin. *Mol Pharmacol* **26**: 99–104, 1984.

27. Hollingworth RM, Fukuto TR and Metcalf RL, Selectivity of sumithion compared with methyl parathion. Influence of structure on anticholinesterase activity. *J Agric Food Chem* **15**: 235–241, 1967.
28. Benke GM, Cheever KL, Mirer FE and Murphy SD, Comparative toxicity, anticholinesterase action and metabolism of methyl parathion and parathion in sunfish and mice. *Toxicol Appl Pharmacol* **28**: 97–109, 1974.
29. Lauwerys RR and Murphy SD, Comparison of assay methods for studying *O,O*-diethyl, *O-p*-nitrophenyl phosphate (paraoxon) detoxication *in vitro*. *Biochem Pharmacol* **18**: 789–800, 1969.
30. Sedmak JJ and Grossberg SE, A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Anal Biochem* **79**: 544–552, 1977.
31. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
32. Hames BD, *Gel Electrophoresis of Proteins: A Practical Approach* (Eds. Hames BD and Rickwood D), p. 1. IRL Press, Oxford, England, 1981.
33. Lu AYH, Levin W and Kuntzman R, Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. *Biochem Biophys Res Commun* **60**: 266–272, 1974.
34. Mackness MI and Walker CH, Multiple forms of sheep serum A-esterase activity associated with the high-density lipoprotein. *Biochem J* **250**: 539–545, 1988.
35. Haas A and Geldmacher-von Mallinckrodt M, Enzyme visualization with partially dehydrated agarose substrate layers: Detection of paraoxonase after thin-layer isoelectric focusing in agarose. *Electrophoresis* **11**: 621–626, 1990.
36. Aldridge WN, Serum esterases 1. Two types of esterase (A and B) hydrolyzing *p*-nitrophenyl acetate, propionate, and butyrate, and a method for their determination. *Biochem J* **53**: 110–117, 1953.