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Identification and Isolation of Two Rat Serum Proteins with A-Esterase Activity toward Paraoxon and Chlorpyrifos-oxon

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ABSTRACT. The active metabolites (oxons) of phosphorothionate insecticides can be detoxified via A-esterase hydrolysis. Two enzymes with A-esterase activity have been isolated from rat serum. Whole serum was applied to anion exchange gel (DEAE Sepharose Fast Flow) and incubated (1 hr). Tris-HCl buffer (0.05 M; pH 7.7, at 5°) containing 0.25 M NaCl was added to the slurry and incubated. The decant, containing low A-esterase activity but a high protein concentration, was discarded. Further displacement of A-esterase from DEAE gel was achieved with 1.0 M NaCl in 0.05 M Tris-HCl buffer (pH 7.7 at 5°). Following desalting and concentration, further separation was achieved by gel filtration (Sephacryl S-100 HR) and two sequential preparative scale isoelectric focusings. Final fractions contained two proteins of high molecular mass (one about 200 kDa and one between 137 and 200 kDa). The apparent range of isoelectric points for the two enzymes was 4.5 to 5.6. Following native-PAGE analysis, activity stains with β-naphthyl acetate and Fast Garnet GBC in the presence of paraoxon (10^{-5} M) verified that A-esterase activity was associated with both proteins. Spectrophotometric assay detected A-esterase activity toward paraoxon, chlorpyrifos-oxon, and phenyl acetate in the final preparation. BIOCHEM PHARMACOL 52;2:363–369, 1996.

KEY WORDS. A-esterase; paraoxonase; paraoxon; chlorpyrifos-oxon; organophosphates

OP§ insecticides or their active metabolites poison insects and non-target animals by inhibiting AChE (EC 3.1.1.7). The phosphorothionates, parathion and chlorpyrifos, are weak anticholinesterases that are activated by cytochromes P450 to the oxon (phosphate) metabolites Pxn and Cpxn. Esterases have been characterized according to their interaction with these compounds, specifically Pxn: (1) A-esterases (also paraoxonase, EC 3.1.1.2) that hydrolyze and, thereby, detoxify paraoxon and are not inhibited by it; (2) B-esterases (such as carboxylesterases, EC 3.1.1.1, and AChE) that degrade Pxn via their phosphorylation and are, thereby, inhibited [1, 2]; and (3) C-esterases that neither affect nor are affected by Pxn [3]. The A-esterases are calcium-activated enzymes that hydrolyze the oxons to their constituent acids and alcohols, thereby detoxifying them.

A-Esterase activity appears to provide protection against OP insecticide exposure. For example, mammals have the highest levels of A-esterase activity, whereas birds, which are more susceptible to OP insecticide toxicity, have little to no activity [3, 4]. Further, rats and mice injected with purified rabbit A-esterase exhibited less OP compound-related toxicity than controls [5, 6]. However, the high K_m of A-esterase toward Pxn [7] suggests that it would be of limited activity in a poisoning by a highly toxic compound such as parathion (rat oral LD_{50}, 3.6 to 13 mg/kg [8]) because only relatively low concentrations of paraoxon would be expected to arise *in vivo*. Our laboratories have reported recently that paraoxon cannot be hydrolyzed *in vitro* at low concentrations ($\leq 10^{-5}$ M) that would be toxicologically relevant [9, 10]. However, *in vitro* hydrolysis at these concentrations has been observed with chlorpyrifos-oxon, the active metabolite of a substantially less toxic insecticide, chlorpyrifos (rat oral LD_{50}, 135–163 mg/kg [8]). This A-esterase activity, which displays very different patterns for these two oxons, may be extremely important in the lower toxicity of chlorpyrifos.

Partial purification of A-esterase from sheep serum suggested the existence of multiple enzyme forms. Gel filtration indicated that proteins with molecular masses of greater than 200 kDa were responsible for paraoxonase activity; however, denaturing electrophoresis revealed a protein that had a molecular mass of 71 kDa [11]. Gel filtration chromatography used to isolate human serum paraoxonase

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§ *Abbreviations:* OP, organophosphorus; AChE, acetylcholinesterase; Pxn, paraoxon, (O,O-diethyl O-4-nitrophenyl phosphate); Cpxn, chlorpyrifos-oxon [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphate]; PA, phenyl acetate; β-NAc, β-naphthyl acetate; PSIEF, preparative scale isoelectric focusing; and HDL, high density lipoprotein.

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activity revealed two paraoxonase activity peaks, one of which coeluted with serum albumin [12]. The researchers reported that albumin will also hydrolyze paraoxon.

Recently, two groups have purified human serum A-esterase, and both describe two enzymes with activity toward Pxn and PA. Gan and colleagues [13] purified two allozymic forms which they designated types A and B. They were unable to distinguish between the two allozymes by SDS–PAGE and reported that each has a molecular mass of 43 kDa. They also reported differences between the allozymes in pH activity profiles, activity response to salts, and turnover rates for Pxn; no difference in turnover rate was apparent for PA or Cpxn [14]. Furlong and coworkers [15] reported purification of human and rabbit serum paraoxonase. Enzymes with molecular masses of 35 and 38 kDa were described in rabbit serum, each of which exhibited activity toward Pxn, PA, and β-NAc. Human serum yielded slightly heavier proteins of 44.7 and 47.9 kDa, which displayed activity toward Pxn, PA, and β-NAc.

Traditionally, PA has been recognized as a substrate for arylesterase. It has been reported that arylesterase(s) and A-esterase(s) are the same enzyme(s) and that this enzyme (or group of enzymes) hydrolyzes PA in addition to Pxn, Cpxn, and β-NAc [13, 15].

In our work with several rat enzymes involved in the metabolism of phosphorothionate insecticides, we have determined that Sprague–Dawley rat serum has high A-esterase activity toward high concentrations of PA (1 mM), Cpxn (0.32 mM) and Pxn (5 mM) and toward low, toxicologically relevant concentrations of Cpxn ($\leq 10^{-5}$ M) [10]. The purpose of this study was to investigate further these protective activities in rat serum by determining: (1) the number of proteins with A-esterase activity, (2) their approximate molecular masses, (3) their estimated isoelectric points, and (4) methods for isolating the proteins as a prelude to further purification and study of the biochemical properties of A-esterase(s) in this species.

MATERIALS AND METHODS

Chemicals

Biochemicals were obtained from the Sigma Chemical Co. (St. Louis, MO) or the Bio-Rad Co. (Hercules, CA). The gel filtration and ion-exchange media were procured from Pharmacia, Inc. (Piscataway, NJ), Metofane™ (methoxyflurane) was purchased from Pitman-Moore, Inc. (Mundelein, IL), and organophosphate compounds were synthesized as described earlier [16].

Animal Care

Male Sprague–Dawley rats [Crl:CD(SD)BR] (200–250 g) were housed at 22 ± 2° in a 12:12 hr light:dark cycle and given tap water and Purina laboratory rat chow *ad lib*. All procedures were approved previously by the Mississippi State University Animal Care and Use Committee.

Tissue Samples

Rats were anesthetized by inhalation of Metofane™ (methoxyflurane). The abdominal cavity was opened, a needle was injected into the vena cava, and blood was collected into a syringe and placed on ice. Clotted blood was then centrifuged (17,000 *g*; 15 min; 4°) to obtain serum. All samples were stored at −70°.

Isolation of Two Rat Serum Proteins with A-Esterase Activity

Whole rat serum (25.0 mL) was added to anion exchange gel (DEAE Sepharose Fast Flow; 500 mL) and incubated for 1 hr in a shaking ice bath. A 450-mL aliquot of 0.05 M Tris-HCl buffer (pH 7.7 at 5°) containing 0.25 M NaCl was added to the slurry and incubated within a shaking ice bath (15 min). The slurry was centrifuged and the supernate, with low A-esterase activity but a high protein concentration, was discarded; this was repeated twice. A 450-mL aliquot of 0.05 M Tris–HCl buffer (pH 7.7 at 5°) containing 1.0 M NaCl was then added to the slurry and incubated in a shaking ice bath (15 min). This was centrifuged, and the supernate (containing A-esterase activity) was desalted and concentrated to 6.0 mL in an Amicon microfiltration device using a PM 30 membrane under pressure of nitrogen gas.

Gel filtration medium (Sephacryl S-100 HR) was packed into a 2.5 × 100 cm Pharmacia column and equilibrated with 0.05 M Tris–HCl buffer (pH 7.7 at 5°) containing 1 mM $CaCl_2$. THe 6.0-mL sample was loaded onto this column and then eluted at a flow rate of 36 mL/hr with equilibration buffer; fractions (2.0 mL) were collected and assayed for A-esterase activity toward Cpxn. Active samples were pooled (an elution volume of 60.0 mL), assayed for activity toward PA, Pxn, and Cpxn, and then divided into two 30.0-mL samples. Ampholytes (2.0 mL; Bio-Rad pH range 3–10) were added to each sample, and each was diluted to 60.0 mL and separately loaded onto a PSIEF unit (Bio-Rad Rotofor) [17].

Once the PSIEF procedures were complete, samples were removed from the PSIEF unit and assayed for pH and A-esterase activity toward Cpxn. The majority of the activity was found in wells 6, 7, and 8 (pH range 5.0 to 6.5). PSIEF wells with peak activity were pooled into a 12.0-mL sample that was diluted to 50.0 mL. Ampholyte solution (2.0 mL; Bio-Rad, pH range 5 to 8) was added, and the sample was diluted to 60.0 mL. This was loaded onto the PSIEF unit.

Once PSIEF was complete, samples were removed from the unit and assayed for pH and A-esterase activity toward Cpxn. Activity was typically found in wells 2 through 6 (pH range 4.5 to 5.6) with the peak wells being 3 and 4 (pH 4.9 and 5.2, respectively). Samples from the active wells were applied to both SDS- and native PAGE gels.

SDS–PAGE

SDS- and native PAGE were conducted with 10% acrylamide resolving gels and 4% acrylamide stacking gels on a

Mini-Protean II Electrophoresis Cell (Bio-Rad) [18]. The gels for SDS–PAGE contained 1% SDS. Samples for SDS–PAGE were diluted in 0.125 M Tris–HCl buffer (pH 6.8) containing 20% glycerol, 0.05% bromphenol blue, and 4% SDS before application to the stacking gel. Some samples and standards were boiled (10 min) in this solution containing 10% (v/v) β-mercaptoethanol before SDS–PAGE analysis. A 0.05 M Tris buffer (pH 8.3) with 0.384 M glycine and 0.2% SDS was used as electrode buffer. Electrophoresis was run at constant voltage (200 V) for 45 min. Electrophoresed gels were stained for protein with Coomassie Brilliant Blue R-250 [19].

Native PAGE

Samples for native PAGE were diluted in 0.062 M Tris–HCl buffer (pH 6.8) containing 10% glycerol and 0.001% bromphenol blue. Pxn was added to a final concentration of 10^{-5} M to inhibit carboxylesterase activity. The electrode buffer was the same as above without SDS. Electrophoresis was run at constant voltage (200 V) for 1 hr. Electrophoresed gels were stained for A-esterase activity by a modification of Furlong *et al.* [15]. Gels were moved into 200 mL of buffer containing 1.0 mM $CaCl_2$ or 1.0 mM EDTA. These gels were incubated in a shaking water bath (37°; 5 min). A 0.375-mL aliquot of 0.2 M β-NAc in ethanol and 2.0% Triton X-100 was added to 100 mL of 0.05% Fast Garnet GBC (or 0.1% Fast Blue RR) in 0.05 M Tris–HCl buffer (pH 7.4 at 37°). This was added to the solution containing the PAGE gels (final concentration of 5.0 mM β-NAc, 0.25% ethanol, and 0.005% Triton X-100 in 0.05 M Tris–HCl buffer, pH 7.4, at 37°). These gels were incubated at 37° until bands appeared (7–15 min), and then gels were removed to a solution of methanol:acetic acid: water (5:1:5; by vol.) for 15 min to stop the reaction. Then the gels were dried.

Direct A-Esterase Assay for Activity toward Cpxn

The direct assay for activity toward high concentrations of Cpxn was modified from Furlong *et al.* [20]. Serum samples (1.0 mL of 5 μL/mL serum) containing either 1 mM EDTA (to correct for non-A-esterase hydrolysis) or 1 mM $CaCl_2$ were incubated at 37° in a shaking water bath (15 min). Cpxn in ethanol was added to initiate the reaction (final concentration of 320 μM). Reaction mixtures were incubated for an additional 15 min and then stopped with 0.5 mL of 2% SDS. Triplicate subsamples were run. Absorbances were read at 315 nm in a spectrophotometer (Perkin Elmer Lambda 5), and the amount of 3,5,6-trichloropyridinol produced was calculated using a molar extinction coefficient of 7.704×10^3 M^{-1} cm^{-1}.

Direct A-Esterase Assay for Activity toward Pxn

The direct assay for activity toward high concentrations of Pxn was modified from Furlong *et al.* [21]. Serum samples (1 mL of 20 μL/mL serum) containing either 1 mM $CaCl_2$ or 1 mM EDTA were incubated at 37° in a shaking water bath (15 min). Pxn in ethanol was added to the samples to initiate the reaction (final concentration, 5 mM). Reaction mixtures were incubated for an additional 15 min and then stopped with 0.5 mL of a mixture of 2% SDS in 2% Tris base to denature protein and alkalinize the sample for 4-nitrophenol detection. Triplicate subsamples were run. Absorbances were read at 400 nm in a spectrophotometer (Perkin Elmer Lambda 5), and the amount of 4-nitrophenol produced was calculated using a molar extinction coefficient of 1.7408×10^4 M^{-1} cm^{-1}.

Direct A-Esterase Assay for Activity toward PA

The assay for activity toward PA was modified from that of Johnson [22]. Serum samples (2.0 mL of 0.25 μL/mL serum) containing either 1 mM $CaCl_2$ or 1 mM EDTA were incubated at 37° in a shaking water bath (15 min). PA in ethanol was added to initiate the reaction (final concentration, 1 mM). The reaction mixtures were incubated for an additional 10 min and stopped with 0.5 mL of 0.4% aminoantipyrine in 5% SDS. An aliquot of 0.25 mL of 1% potassium ferricyanide was added to develop color. Triplicate subsamples were run. Absorbances were read at 510 nm in a spectrophotometer (Perkin Elmer Lambda 5), and the amount of phenol produced was calculated using a molar extinction coefficient of 1.4162×10^4 M^{-1} cm^{-1}.

Indirect A-Esterase Assay for Activity toward Cpxn

The indirect assay of activity toward low concentrations of Cpxn and Pxn (10^{-6}–10^{-8} M) was modified from Chambers *et al.* [9], reducing the volumes to one-fourth of those described. 4-Nitrophenyl diphenylphosphinate was added to each sample (final concentration of 0.67 μM) and allowed to incubate at 4° for 10 min before assay in order to inhibit carboxylesterases. The indirect assay assessed the residual oxon not hydrolyzed by the A-esterases by its ability to inhibit an exogenous source of AChE. This method allowed detection of A-esterase activity at substrate concentrations well below the detection limits of direct spectrophotometric methods.

Protein Assay

Protein concentrations were determined in triplicate, using bovine serum albumin as the standard by the method of Lowry *et al.* [23].

RESULTS

High A-esterase activities toward high concentrations of PA, Cpxn, and Pxn (as measured by the direct assay) and toward low, toxicologically relevant concentrations of Cpxn (as measured by the indirect assay) were detected in rat serum (Table 1). Native PAGE of whole rat serum

TABLE 1. A-Esterase activities in rat serum toward phenyl acetate, chlorpyrifos-oxon, and paraoxon

Substrate	Direct assay		Indirect assay	
	Serum (μL/mL)	Activity (nmol product formed · mg protein^{-1} · min^{-1})	Serum (μL/mL)	Activity (pmol product formed · mg protein^{-1} · min^{-1})
Phenyl acetate	0.25	388.4 ± 29.8	*	*
Chlorpyrifos-oxon	5.0	36.7 ± 1.7	20.0	602.5 ± 30.4
Paraoxon	20.0	4.9 ± 0.4	20.0	Not detected

Values are means ± SEM, N = 4.
* Activities toward phenyl acetate were not assayed indirectly.

produced two closely migrating, high molecular mass proteins that stained for A-esterase activity throughout the purification process with Fast Garnet GBC (and Fast Blue RR) in the presence of β-NAc and 10^{-5} M Pxn (noted by arrows, Fig. 1). The fractions containing these two proteins exhibited A-esterase activity toward Cpxn, Pxn, and PA. Serum albumin also stained under these conditions (Fig. 1), indicating potential oxon hydrolysis capability. Albumin would also stain with Fast Garnet GBC in the absence of the substrate β-NAc, but the bands were more intense in its presence.

The final purification process yielded two PSIEF samples (wells 1 and 2; pH 4.4 and 4.5, respectively) containing two major protein bands that stained in the presence of Coomassie Brilliant Blue (Fig. 2, lanes 3 and 4). These samples also contained two proteins that stained for A-esterase activity (noted by arrows, Fig. 3). Other PSIEF samples (wells 3, 4, and 5; pH 4.9, 5.2, and 5.5, respectively) contained these same two proteins; however, they also contained a third polypeptide that stained with Coomassie Brilliant Blue and the A-esterase staining procedure (Fig. 1, lanes 1–4, and Fig. 2, lanes 5–7). This polypeptide comigrated with bovine serum albumin standards (Fig. 2).

The molecular masses of the serum A-esterase proteins appeared to be above 200 kDa according to SDS–PAGE analysis without β-mercaptoethanol (Fig. 2). Samples boiled for 10 min with β-mercaptoethanol before SDS–PAGE analysis still revealed two protein bands; however, the molecular mass of the proteins appeared slightly lower (Fig. 4). The heavier protein migrated at about 200 kDa,

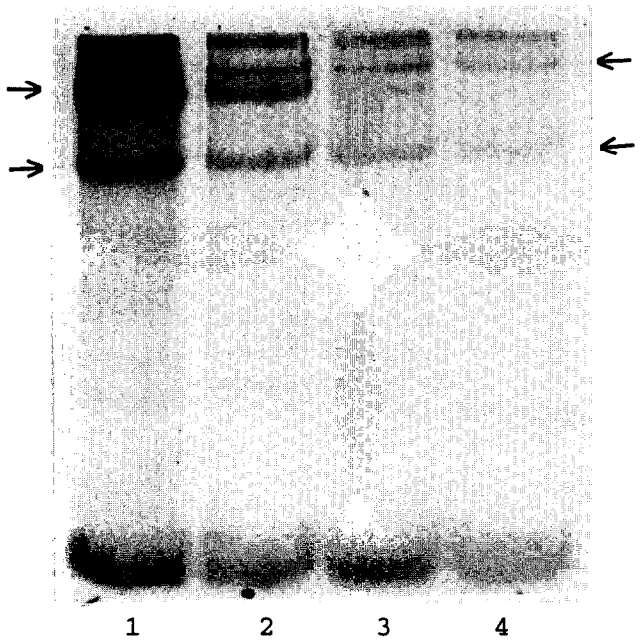

FIG. 1. Native PAGE gel of rat serum fractions from purification stained for A-esterase activity using β-NAc and Fast Garnet GBC. (Arrows indicate A-esterase activity.) Lane 1 is a sample after Sephacryl S-100 gel filtration. Lane 2 is a pooled sample from the first PSIEF run (ampholyte pH range 3–10). Lane 3 is well 4 (pH 5.2) from the second PSIEF run (ampholyte pH range 5–8). Lane 4 is well 3 (pH 4.9) from the second PSIEF run (ampholyte pH range 5–8).

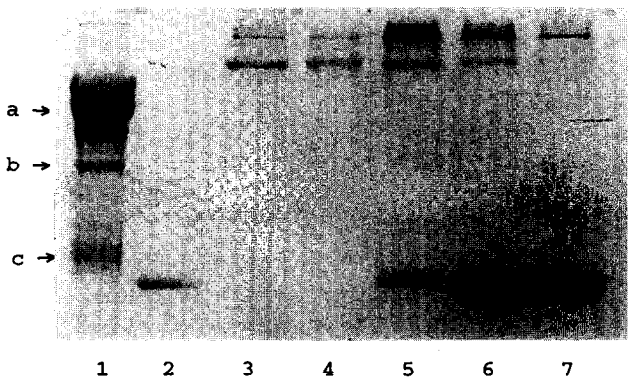

FIG. 2. SDS–PAGE gels of rat serum fractions stained for proteins with Coomassie Brilliant Blue. Lane 1 contains the following broad spectrum molecular weight standards (Bio-Rad): a = myosin, 206 kDa; b = β-galactosidase, 137 kDa; and C = bovine serum albumin, 83 kDa. Lane 2 is bovine serum albumin standard (Sigma), molecular mass = 66.43 kDa. (The bovine serum albumin standards were obtained from different suppliers and, therefore, have different molecular masses.) Lane 3 is well 1 (pH 4.4) from the second PSIEF (ampholyte pH range 5–8). Lane 4 is well 2 (pH 4.5) from the second PSIEF (ampholyte pH range 5–8). Lane 5 is well 3 (pH 4.9) from the second PSIEF (ampholyte pH range 5–8). Lane 6 is well 4 (pH 5.2) from the second PSIEF (ampholyte pH range 5–8). Lane 7 is well 5 (pH 5.5) from the second PSIEF (ampholyte pH range 5–8). (Arrows indicate proteins staining with Coomassie Brilliant Blue.)

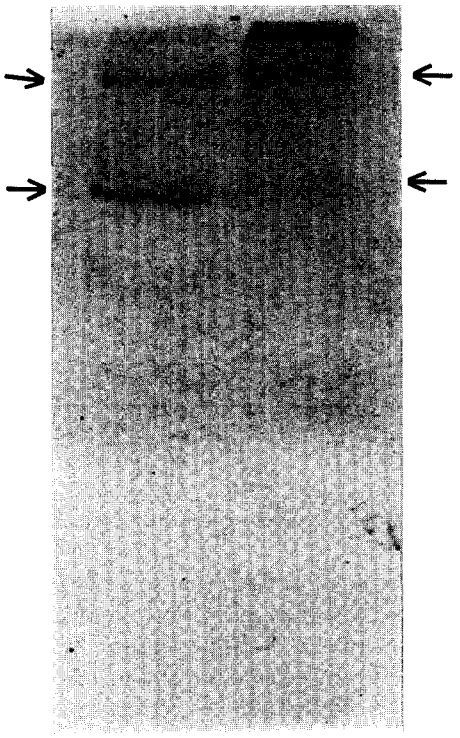

FIG. 3. **Native-PAGE gel of serum fractions from the second PSIEF stained for A-esterase activity using β-NAc and Fast Garnet GBC. (Arrows indicate A-esterase activity). Lane 1 is well 2 (pH 4.5) from PSIEF (ampholyte pH range 5–8). Lane 2 is well 1 (pH 4.4) from PSIEF (ampholyte pH range 5–8).**

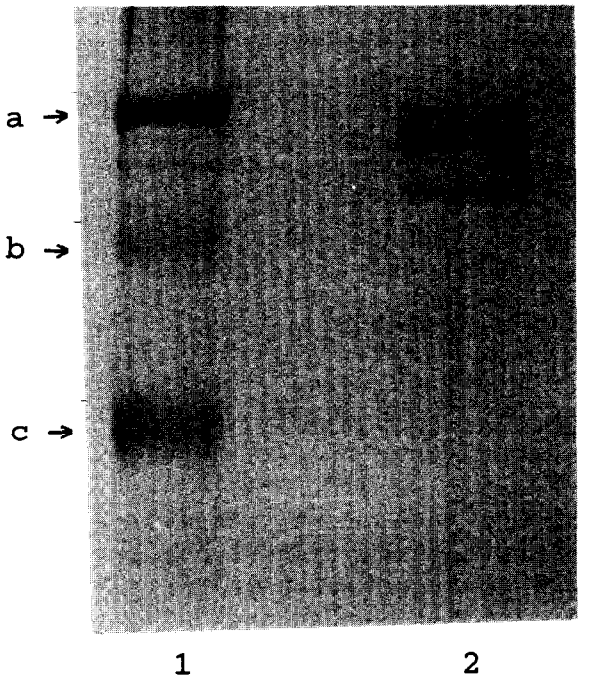

FIG. 4. **SDS–PAGE gel of serum fraction from the second PSIEF (well 2). (Arrows indicate proteins staining with Coomassie Brilliant Blue.) Lane 1 contains the following broad spectrum molecular weight standards (Bio-Rad): a = myosin, 206 kDa; b = β-galactosidase, 137 kDa; and c = bovine serum albumin, 83 kDa. Lane 2 is well 2 (pH 4.5) from PSIEF (ampholyte pH range 5–8) treated with β-mercaptoethanol.**

while the second protein migrated between standards of 200 and 137 kDa. The isoelectric point was in the range of 4.5 to 5.6.

Samples from the final PSIEF (wells 1 and 2 pooled; wells 3, 4, and 5 pooled) contained the two proteins that stained for A-esterase activity on native PAGE gels and also exhibited hydrolytic activity toward PA, Cpxn, and Pxn (Table 2). The serum purification table demonstrates that the percent yield of A-esterase was very low (Table 3). The PSIEF appeared to negatively affect the activity of the A-esterase proteins. The specific activity was lowered considerably by this procedure (Table 3). Yield was sacrificed to produce high purity.

DISCUSSION

The whole serum A-esterase activities using the direct assays were about 6–7 times higher toward Cpxn than toward Pxn, using approximately 16-fold less Cpxn as substrate. This suggests that rat serum A-esterases can degrade Cpxn more readily than Pxn as has been reported with human serum A-esterases [20]. Occurrence of activity toward low, toxicologically relevant concentrations of Cpxn ($\leq 10^{-5}$ M) and absence of activity toward similar concentrations of Pxn [9] also suggest that A-esterase(s) is able to degrade Cpxn more readily than Pxn. The absence of activity toward these low Pxn concentrations may be attributable to differences in affinity for various substrates by the two isozymes. Perhaps, as previously suggested [21], the isozymes have equally high affinities for Cpxn, whereas only one has a high affinity for Pxn so that the activity toward low Pxn concentrations is not detectable. Alternately, the A-esterase may simply have an undetectably low velocity at low Pxn concentrations.

Two protein bands that stained for A-esterase activity toward β-NAc were isolated from rat serum into a sample that exhibited hydrolysis of PA, Cpxn, and Pxn. (The heavier protein consistently stained more strongly than the lighter one.) It appears, therefore, that both of these are A-esterases. These data correlate well with other literature

TABLE 2. **A-Esterase specific activities of pooled samples from a representative final preparative scale isoelectric focusing procedure (ampholyte pH range 5–8)**

	Specific activity (nmol product formed · mg protein^{-1} · min^{-1}) Substrates		
PSIEF well no.	Phenyl acetate	Chlorpyrifos-oxon	Paraoxon
1 and 2	52.1	15.7	4.9
3, 4, and 5	30.4	11.3	3.8

TABLE 3. Purification of A-esterase from rat serum

Fraction	Volume (mL)	Total activity (nmol/min)	Total protein (mg)	Specific activity* (nmol product formed · mg protein^{-1} · min^{-1})	% Yield
Crude serum	25.0	239091.6	1505.0	158.9	
Batch DEAE	6.0	31276.6	272.0	115.0	13.1
Gel filtration	60.0	22429.4	96.0	233.6	9.4
PSIEF (ampholyte pH 3–10; wells 6–8)	12.5	8478.4	29.6	286.4	3.5
PSIEF (ampholyte pH 5–8; wells 1 and 2)	4.6	414.4	5.9	70.8	0.17
PSIEF (ampholyte pH 5–8; wells 3–5)	6.2	1997.1	36.9	54.1	0.84

* Specific activity toward chlorpyrifos-oxon.

reporting the existence of two isozymes in rabbit and human sera [13, 15]. As is the case with the proteins of these reports, the two proteins here are very similar to one another in molecular mass and isoelectric point. Also, concurring with previously reported data [15, 20, 21], the proteins isolated here appear to hydrolyze β-NAc, PA, Cpxn, and Pxn, suggesting that they possess both A-esterase and arylesterase activities.

The molecular masses reported here (one about 200 kDa and a second between 200 and 137 kDa; Fig. 4, lane 2) are considerably higher than the molecular masses reported for A-esterase from human or rabbit serum [13, 15]. Since serum A-esterases have been reported to be associated with the HDL complex [24], it is possible that portions of this complex are still adhering to the proteins of interest, thereby resulting in high molecular masses. Perhaps the rat A-esterase–HDL complex is more resistant than those of other species to hydrolysis or other forms of degradation. Also, it may be that rat A-esterases are actually larger than those of other species. Perhaps alternate splicing of mRNA, which produces larger transcribed proteins, occurs. The range of isoelectric points that we report concurs with the pI value of 5.1 reported previously [13].

The third band that appeared in the Coomassie-stained SDS–PAGE analysis of PSIEF wells 3, 4, and 5 (Fig. 2, lanes 5–7) is believed to be serum albumin. The protein comigrated on SDS–PAGE with bovine serum albumin standards (Fig. 2) and stained for A-esterase activity (Fig. 1). This concurs with reports that serum albumin possesses A-esterase activity [25]. This protein was extremely difficult to remove and prevented a high yield recovery of pure A-esterase.

Our yield of A-esterase was low. A great deal of A-esterase activity was lost with the batch anion exchange procedure. Perhaps the recovery could be improved with replacement of this step by gradient elution of protein fractions from an anion-exchange column. We have been unable to separate the two A-esterase proteins by means other than electrophoresis; therefore, we have not obtained enough of the separated proteins for kinetic analysis. Improvement of recovery could perhaps allow us to electroelute the proteins from native-PAGE gels and thereby separate them.

In conclusion, two enzymes exist in rat serum which stain for A-esterase activity toward β-NAc and are very similar in terms of molecular mass and isoelectric point. These two enzymes have been isolated into a sample that exhibits hydrolysis of PA, Cpxn, and Pxn. It appears likely that rat serum A-esterase and arylesterase activities may be attributable to the same proteins.

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