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PARTIAL CHARACTERIZATION OF AN ENZYME THAT HYDROLYZES SARIN, SOMAN, TABUN, AND DIISOPROPYL PHOSPHOROFLUORIDATE (DFP)*

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Abstract—The properties of a rat liver enzyme that hydrolyzes organophosphorus (OP) inhibitors of cholinesterases were studied. The rates of hydrolysis of OP inhibitors were determined by continuous titration of released hydrogen ions, using a pH stat method. Centrifugation of homogenates at 205,000 *g* for 30 min demonstrated that the activity was in the soluble fraction. Hydrolysis of sarin, soman, and diisopropyl phosphorofluoridate (DFP), but not of tabun, was stimulated by the addition of Mn^{2+} and Mg^{2+}. Hydrolysis of sarin > soman > tabun > DFP. Unlike other OP hydrolases that preferentially hydrolyze the non-toxic isomers of soman, this enzyme hydrolyzed all four soman isomers at approximately the same rate. This result was obtained *in vitro* by gas chromatographic analysis of enzyme-catalyzed soman hydrolysis and confirmed *in vivo* by demonstrating reduced toxicity in mice of soman partially hydrolyzed by this enzyme. K_m and V_{max} were determined by fitting V vs $[S]$ to a hyperbolic function using regression analysis. K_m values ranged from 1.1 mM for soman to 8.9 mM for tabun. V_{max} values ranged from 54 nmol/min/mg protein for DFP to 2694 for sarin. The enzyme was stable for at least 2 months at −90° but was inactivated by heating at 100° for 5 min. Elution profiles from gel filtration by high pressure liquid chromatography showed that the hydrolytic activity for the OP inhibitors eluted in a single peak, suggesting that a single enzyme was responsible for the observed hydrolysis. Further purification and characterization of this enzyme should prove useful for the development of methods for detection, detoxification, and decontamination of these cholinesterase inhibitors.

The highly toxic nature of organophosphorus compounds has resulted in their development as insecticides and chemical warfare agents. By irreversibly inhibiting acetylcholinesterase, an enzyme critical to the normal functioning of the nervous system, organophosphorus compounds produce a variety of toxic effects [1]. Acetylcholinesterase normally hydrolyzes the neurotransmitter acetylcholine at the synaptic junction, thus terminating neuronal transmission [1]. The inhibition of acetylcholinesterase leads to a large and rapid accumulation of acetylcholine at the synaptic junction, resulting in uncontrolled neuronal stimulation and subsequent blockade of neuronal function [1]. The net result of such a blockade is manifested in signs such as bradycardia, pulmonary constriction, increased bronchial secretions, muscle paralysis, seizures, and eventually death from respiratory failure [2–4].

We describe here the partial characterization of an enzyme, in mammalian liver, that catalyzes the hydrolysis of diisopropyl phosphorofluoridate (DFP) and also of the chemical warfare nerve agents isopropyl methylphosphonofluoridate (sarin), ethyl-*N*-dimethyl phosphoramidocyanidate (tabun) and pinacolyl methylphosphonofluoridate (soman). Soman contains two chiral centers and therefore exists as four stereoisomers [5, 6]. Two of these isomers are extremely toxic and rapidly inhibit acetylcholinesterase, whereas the other two isomers are poor inhibitors and are considered essentially non-toxic [5, 7]. Previously reported organophosphorus hydrolases preferentially hydrolyze the non-toxic soman isomers [6, 8–10]. The enzyme reported here not only hydrolyzes all three nerve agents at a rate significantly greater than do previously reported enzymes, but also is the first example of an enzyme that hydrolyzes the extremely toxic isomers of soman at the same rate as the non-toxic isomers [8, 10, 11].

MATERIALS AND METHODS

Chemicals

Soman, sarin, and tabun were obtained from the Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD. Analysis by nuclear magnetic resonance spectroscopy showed tabun to be 85.8% pure, sarin to be 96.6% pure, and soman to be 99.1% pure. DFP was obtained from

* The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* (1985), as prepared by the Committee on Care and Use of Laboratory Animals, National Research Council, NIH Publication No. 85-23.

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ICN Pharmaceutical, Plainview, NY. Horseradish peroxidase and bovine alkaline phosphatase, Type XX-T, were obtained from Worthington, Freehold, NJ. All other chemicals were analytical grade.

Animals

Male rats (200–250 g) of the Sprague–Dawley stock [Crl:CD(SD)BR] and male mice (28–32 g) of the ICR stock [Crl:CD-1(ICR)BR] were obtained from Charles River (Kingston, NY) and maintained on lab chow and tap water *ad lib*. All animals were maintained on a 12-hr day–night cycle (light cycle from 6:00 a.m. to 6:00 p.m.). Rats were fasted for 18 hr before they were used in experiments. Fasting was utilized to increase the yield of hepatic subcellular fractions [12] prepared from the same liver homogenate but used for other purposes. Subsequent experiments have demonstrated that fasting had no effect on the activity of the enzyme(s) characterized in this manuscript.

Preparation of the soluble cell fraction

Rat livers were removed, placed in beakers on ice, rinsed with ice-cold 0.25 M sucrose, minced with scissors, and then placed in 3 vol. of ice-cold 0.25 M sucrose. They were then homogenized (12 strokes at 400 rpm), using a mechanically driven Teflon pestle in a glass homogenizer (Arthur H. Thomas, Inc., Philadelphia, PA). After diluting the homogenate to 5% (w/v) with 0.25 M sucrose, nuclei and mitochondria were removed by successive centrifugation at 1,000 *g* for 10 min and 10,000 *g* for 10 min in a Sorvall RC-5B refrigerated centrifuge (DuPont Co., Wilmington, DE). The post-mitochondrial supernatant fraction was then centrifuged at 205,000 *g* for 30 min in a Beckman SW41 rotor operated in a Beckman model L8-80 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). It should be noted that the soluble fraction is usually prepared by centrifugation at 105,000 *g* for 60 min. On the basis of protein recovery and preliminary enzyme analysis, we consider our soluble fraction to be essentially identical. The supernatant fraction was collected and is referred to as the soluble fraction.

Determination of hydrolysis rates of organophosphorus compounds

The rate of hydrolysis of each substrate was measured using an automated Radiometer pH Stat system (Radiometer America, Cleveland, OH), by continuously titrating with a previously standardized 0.01 N KOH solution the acid generated upon hydrolysis. Titrations were carried out at pH 7.4 and 25° in unbuffered isotonic saline containing the substrate and metal ion concentrations indicated in the legends to the tables and figures. For all assays, 3.0 ml of a substrate solution in saline was introduced into the titration cell, and the rate of spontaneous hydrolysis was determined. For enzyme assays, 25–100 μl of soluble fraction containing 1–2 mg of protein was then introduced into the substrate solution to obtain the total hydrolysis rate. The enzymatic hydrolysis rate was then calculated by subtracting the spontaneous rate from the total rate.

Gel filtration by high pressure liquid chromatography (HPLC)

To 1.0 ml of the soluble fraction were added 200 units of bovine intestinal alkaline phosphatase and 10 units of horseradish peroxidase to act as marker enzymes in the elution process. The mixture was filtered through a Millex-GV 0.22 μm filter unit (Millipore Corp., Bedford, MA), and 50 μl of the filtrate was injected onto a type G2000SW TSK-GEL column (Toyo-Soda, Japan) and eluted at a flow rate of 1.0 ml/min with 0.01 M NaCl adjusted to pH 7.0. Aliquots (1 ml) were collected, and each aliquot after the void volume (10.75 min) was assayed for alkaline phosphatase and horseradish peroxidase activity as well as for hydrolyzing activity against sarin, soman, tabun, and DFP.

Measurement of enzyme activity in HPLC gel column fractions

Organophosphorus-hydrolyzing activity. To 1.0 ml of 10 mM sarin, soman, tabun, or DFP were added 1.0 ml of a 2 mM $MgCl_2$ solution and 200 μl of methyl red (1 mg/ml in ethanol). Fifty microliters of this "substrate" solution was pipetted into each of 24 wells of a standard microtiter plate, each well containing 50 μl of the fraction to be tested. After 2, 10, and 30 min, the plate was read at 510 nm in a Titertek Multiscan MCC (Flow Laboratory Inc., McLean, VA). Absorbances were plotted versus fraction number to determine the elution volume of the enzyme.

Alkaline phosphatase activity. Alkaline phosphatase activity was determined spectrophotometrically as described above, except that 37 mg/ml of 4-nitrophenylphosphate in 1.0 M tris (hydroxymethyl) aminomethane (Tris)–chloride buffer (pH 8.0) was employed as the substrate and a 405 nm filter was used in the Titertek.

Horseradish peroxidase activity. Horseradish peroxidase activity was determined in a manner similar to that described above, except that a fresh solution of 3,3′-diaminobenzidene, 50 μg/ml in 0.1 M acetate buffer (pH 5.0) containing 0.1 μl of 30% hydrogen peroxide/ml, was used as the substrate. Appearance of color was determined by visual observation, and the horseradish peroxidase elution position was verified in a separate experiment by UV detection at 280 nm.

Separation of soman stereoisomers

The stereoisomers of soman were separated by gas chromatography by a modification [13] of the method of Benschop *et al.* [9]. The carbowax-20 capillary column was omitted, and the samples were separated on a Chirasil-val capillary column (25 m × 0.22 mm) (Chrompack, Inc., Bridgeport, NJ) and detected with an electron capture nitrogen/phosphorus detector. All other conditions of column temperature and flow rates were as described by Benschop *et al.* [9].

Bioassay for determining the ability of the liver enzyme to hydrolyze the toxic soman isomers

Five milliliters of 2 mM soman in saline was introduced into the titration cell of the automated Radi-

ometer pH Stat system (see above under "Determination of hydrolysis rates of organophosphorus compounds"). To this was added a 50-μl aliquot of the soluble fraction containing the organophosphorus-hydrolyzing enzyme. The hydrolysis was followed by monitoring base uptake to a volume of base equivalent to 50% hydrolysis of the soman. At that point an aliquot of the 50% hydrolyzed soman was removed, tested for extent of hydrolysis by gas chromatography, and diluted with saline to 40 μg/ml. To another 5 ml of 2 mM soman was added a 50-μl aliquot of heat-inactivated soluble fraction (5 min at 100°). After an equivalent time period this was diluted with saline to 19 μg/ml. A 2 mM soman control that had been stored on ice was also diluted with saline to 19 μg/ml. Three sets of mice were injected in the tail vein with 0.28 to 0.32 ml (10 μl/g) of the partially hydrolyzed soman, the soman that had been incubated with the heat-inactivated soluble fraction, or the control soman. The 24-hr LD_{50} values were determined by plotting the dose in μg/kg versus the percent of mice in each group that died. Six different dilutions were used for each LD_{50} determination with six mice in each group.

Protein assay

Protein was assayed by the method of Lowry *et al.* [14] using bovine serum albumin as the standard.

RESULTS

When liver was homogenized and separated by ultracentrifugation into particulate and soluble fractions, 85% of the soman-hydrolytic activity was observed in the soluble fraction (Table 1). The yield of the soluble fraction was 58 ± 0.8 mg protein/g wet liver weight.

The hydrolytic activity of the liver enzyme was determined using several organophosphorus compounds as substrates (Table 2). Sarin, soman, tabun, and DFP were all hydrolyzed, with sarin being hydrolyzed at the greatest rate. As shown in Table 2, hydrolysis rates were sarin > soman > tabun > DFP. The Michaelis–Menten constant (K_m) and the maximum velocity (V_{max}) of the enzyme with different substrates are also shown in Table 2. The K_m, which is inversely proportional to the affinity of the enzyme for the substrate, was in the millimolar range for soman, sarin, tabun, and DFP. The maximum hydrolytic rate of the enzyme (V_{max}) ranged from a high of 2694 nmoles of substrate hydrolyzed/min/mg of protein with sarin to a low of 54 with DFP.

Table 1. Cellular distribution of the liver organophosphorus-hydrolyzing enzyme

Fraction	Percent of original homogenate activity
Particulate	15 ± 1
Soluble	85 ± 3

Two percent liver homogenates (w/v) in 0.25 M sucrose were sedimented at 205,000 *g* for 30 min. Supernatant fractions were decanted and saved. Pellets were washed once by suspension recentrifugation in 0.25 M sucrose containing 20 mM Tris–Cl (pH 7.5) to remove absorbed soluble proteins [15] and subsequently suspended in 0.25 M sucrose. Washed pellets and soluble fractions were assayed as described in Materials and Methods with 1 mM soman and 1 mM $MgCl_2$. Recoveries were 100 ± 9%. Values are mean ± SE (N = 6).

Table 2. Substrate specificity and kinetic properties of the liver organophosphorus-hydrolyzing enzyme

Substrate	K_m (mM)	V_{max} (nmol/min/mg protein)
Sarin	1.9	2694
Soman	1.1	718
Tabun	8.9	586
DFP	3.7	54

The soluble fractions of six liver homogenates were prepared, combined, and assayed as described in Materials and Methods in the presence of 1 mM $MgCl_2$. Five different substrate concentrations were assayed for each substrate, and the K_m and V_{max} were determined by fitting velocity vs substrate concentration to a hyperbolic function using regression analysis [16]. Results for each substrate, which usually did not differ by more than 10% for K_m or 20% for V_{max}, are those combined from at least two different kinetic experiments.

Since metal ions are known to stimulate the activity of other organophosphorus-hydrolyzing enzymes [17], we studied the effects of Mn^{2+}, Mg^{2+}, and Co^{2+} on the activity of the liver enzyme (Fig. 1). All three ions stimulated the activity of the enzyme when sarin or soman was employed as a substrate, with Mg^{2+} eliciting the largest effect. When tabun was employed as a substrate, these metal ions had no effect on enzyme activity. When DFP was employed as the substrate, Mn^{2+} and Mg^{2+} stimulated the activity to about the same level, whereas Co^{2+} had no effect.

Since Mg^{2+} was shown to stimulate the activity of the enzyme to a greater extent than Mn^{2+} or Co^{2+} when sarin or soman was employed as substrate, the effect of various concentrations of Mg^{2+} on enzyme activity was determined (Fig. 2). All concentrations of Mg^{2+} tested were shown to stimulate enzyme activity, with the greatest degree of stimulation

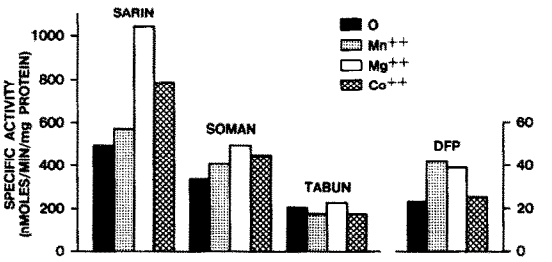


Fig. 1. Effect of metal ions on enzyme activity. Homogenates from six livers were pooled, and the soluble fraction was prepared as described in Materials and Methods. This soluble fraction was assayed for enzymatic activity with a 5 mM concentration of the indicated substrate and in the presence or absence of a 1 mM concentration of the indicated metal ion. Results are shown from one representative experiment. Results from three different experiments did not usually differ by more than 20%.

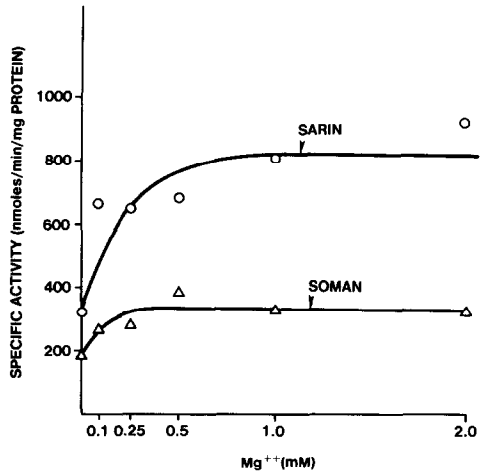


Fig. 2. Effect of Mg^{2+} on the hydrolysis of sarin and soman by the liver enzyme. Homogenates from six livers were pooled, and the soluble fraction was prepared as described in Materials and Methods. The activity of this fraction was determined with 1 mM sarin or soman as the substrate as a function of the Mg^{2+} concentration. Results are shown from one representative experiment.

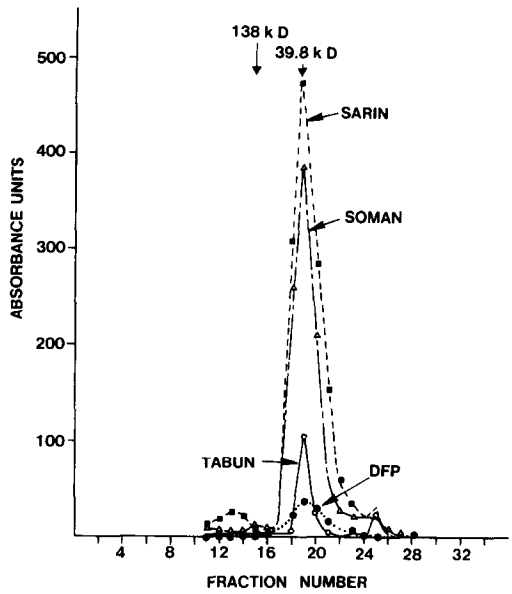


Fig. 3. Molecular weight determination of the liver enzyme. This figure shows elution patterns of the organophosphorus-hydrolyzing enzyme from a 7.5 × 30 mm G 2000 SW type TSK gel HPLC column. The column was run, and the enzymatic activity was determined as described in Materials and Methods. Molecular weight marker enzymes of 138 kD (alkaline phosphatase) and 39.8 kD (horseradish peroxidase) eluted at the indicated positions. Substrates are sarin (■), soman (△), tabun (○), and DFP (●). Results are shown from one representative experiment.

occurring at 1.0 mM. As a result of this experiment, subsequent experiments were conducted in the presence of 1 mM Mg^{2+}. At Mg^{2+} levels higher than 5 mM, significant non-enzymatic hydrolysis was observed, presumably due to Mg^{2+} catalysis [18, 19].

Elution profiles from high pressure liquid chromatography (HPLC) gel filtration columns indicated that a single enzyme was responsible for the hydrolysis of sarin, soman, tabun, and DFP since the hydrolytic activity eluted from the column in a single peak (Fig. 3). These results also indicate that the molecular weight of the enzyme is approximately 40 kD since the enzyme activity co-eluted with that of horseradish peroxidase, which has a molecular weight of 39.8 kD (Fig. 3).

Due to the presence of a center of asymmetry in both the pinacolyl moiety and the phosphorus atom, four steroisomers of soman exist. When soman was hydrolyzed by the liver enzyme for various lengths of time as indicated in the legend of Fig. 4, all four stereoisomers of soman were hydrolyzed at about the same initial rate. The time required for 50% hydrolysis was identical for each of the four stereoisomers.

To verify that the liver organophosphorus-hydrolyzing enzyme hydrolyzes the toxic isomers of soman, mice were injected intravenously with soman, soman that had been incubated with heat-inactivated enzyme, or soman that had been hydrolyzed to the 50% point with the liver enzyme. As shown in Table 3, the LD_{50} in mice for soman was 141 μg/kg. When mice were injected with soman that had been partially hydrolyzed, the LD_{50} increased by about 50% to 212 μg/kg. In other experiments the LD_{50} was increased to the value expected if all isomers were hydrolyzed at the same rate, but the confidence limits were not as satisfactory as in this experiment.

On the basis of a preliminary experiment, it appeared that this enzyme was stable for at least 80 days at −90° and was completely inactivated when boiled for 5 min.

DISCUSSION

We have described an enzyme, isolated from the soluble fraction of mammalian liver, that catalytically hydrolyzed sarin, soman, tabun, and DFP. This enzyme hydrolyzed these toxic compounds at rates significantly greater than those previously reported for organophosphorus-degrading enzymes [11, 21–

Table 3. Effect of partial soman hydrolysis on *in vivo* toxicity in mice

Challenge solution*	LD_{50}† (μg/kg)
Soman	141 (>133 < 150)
Soman + heat-inactivated enzyme	136 (120–153)
Soman + enzyme	212 (195–231)

* Given intravenously to adult males. Each group (soman, soman + heat-inactivated enzyme, and soman + enzyme) contained thirty mice. These thirty mice were then divided into five groups of six mice in each group to which various doses of soman were given.

† Mean with values in parentheses representing the confidence limits determined according to Thompson and Weil [20].

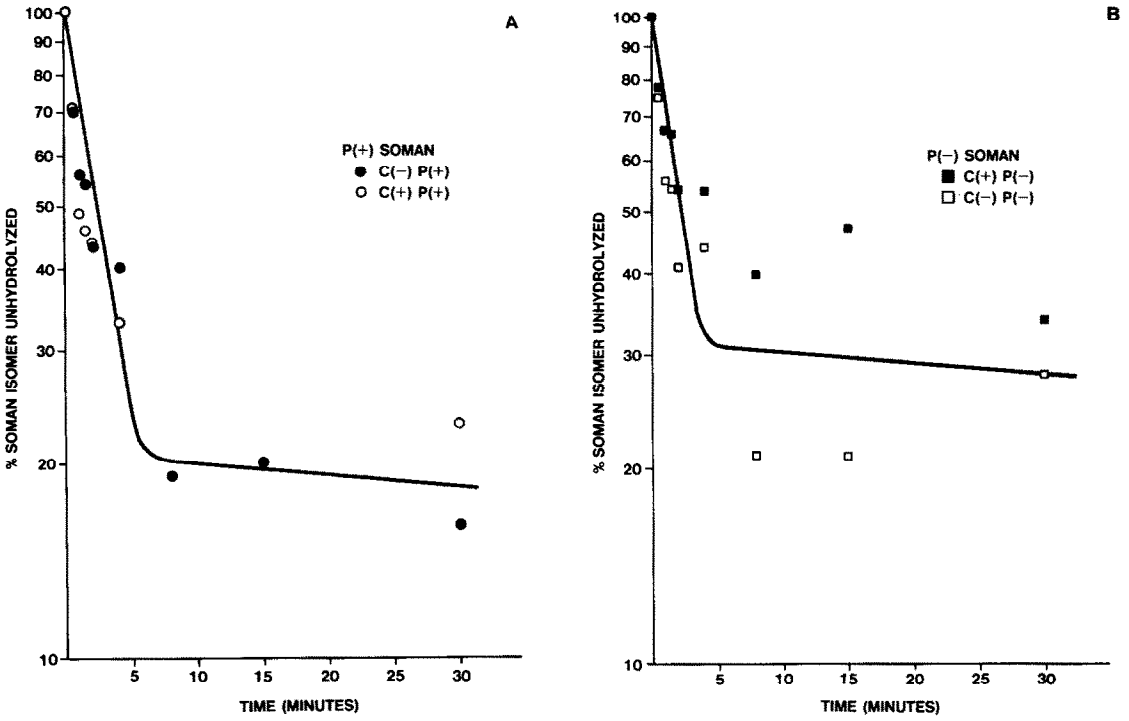


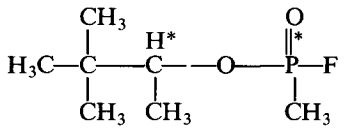
Fig. 4. Hydrolysis of the four soman stereoisomers by the rat liver enzyme. Stereoisomers were separated as described in Materials and Methods. All data are expressed as the percent of a DFP internal standard and have been normalized for direct comparison. All points are the average of two determinations. (A) Hydrolysis of the P(+) soman isomers; and (B) Hydrolysis of the P(−) soman isomers. The C(+)P(−) soman isomers at 8 and 15 min have large values because the area attributable to the C(+)P(+) soman peak was not resolved at these time points and is probably included in the area for the C(+)P(−) soman peak (which is the adjacent peak).

24]. In addition, the soluble enzyme reported here was stimulated most efficiently by Mg^{2+} (Fig. 1), whereas previously reported organophosphorus hydrolases have been stimulated most efficiently by Mn^{2+} [11, 22]. Our results contrast with those of Mounter [17], who found that Mn^{2+} increased the activity of a soluble rat liver fraction by a factor of 7–8, whereas Mg^{2+} had almost no measurable effect, using DFP as a substrate. It is possible that the method used by Mounter, together with the use of DFP as a substrate, required a sufficiently large aliquot of tissue sample such that the endogenous Mg^{2+} maximally stimulated the enzyme so that the effect of additional Mg^{2+} was not observed. If Mg^{2+} is present in rat liver at a concentration of 0.019% [25], it may be calculated that the Mg^{2+} in Mounter's reaction vessel (before the addition of exogenous salt) was approximately 0.1 mM. Our results (Fig. 2) indicate that most of the metal ion effect was manifest at 0.1 mM with soman or sarin as substrates; the same was observed with DFP as substrate (data not shown). Under the conditions of our assay, the endogenous Mg^{2+} from the tissue would be only about 0.01 mM, well below the maximal concentration for stimulation by Mg^{2+}. This could explain the differences observed with Mg^{2+}. The disparity with Mn^{2+} is not so easily resolved. Using DFP as a substrate, Mn^{2+} was slightly more effective at stimulating activity than Mg^{2+}, in contrast to the case with soman or sarin as substrate (Fig. 1). However, in none of our observations have we seen the differences in magnitude of effect that were reported by Mounter. Even with sarin as substrate (in which the largest effects were seen) the activity was stimulated only by about a factor of two.

This enzyme was localized almost exclusively in the soluble fraction of the liver (Table 1), a characteristic that should prove useful in the purification of this enzyme since the use of detergents, which is often necessary to solubilize membrane-bound enzymes, is not required. Although it is possible that the origin of this enzyme(s) is from the particulate fraction, this seems unlikely since the liver was homogenized in 0.25 M sucrose which is known not to solubilize other particulate enzymes such as glucose-6-phosphatase [26, 27] and cytochrome oxidase [28] from liver homogenates. While it is possible that several different enzymes in the soluble fraction of the liver may be responsible for the observed hydrolytic activity against these organophosphorus compounds, this seems unlikely since the hydrolytic activity eluted from gel columns by HPLC in a single peak (Fig. 3). This observation suggests that only one enzyme is responsible for all of the observed hydrolytic activity. This being the case, it is a little puzzling why the metal-ion effects appear to be substrate dependent. There is little doubt that Mg^{2+} was a more effective stimulator than Mn^{2+} when sarin

was the substrate, but was no better, or perhaps even less effective than Mn^{2+} with DFP as substrate. Since the stimulation is the result of a metal ion–protein interaction rather than a metal ion–substrate interaction [29], the observed differences probably reflect very subtle changes in protein conformation due to metal ion binding; such changes could very well be dependent on which metal ion was binding. Since the enzyme is found almost exclusively in the soluble fraction of the liver and is eluted as a single peak from HPLC gel columns, a partial purification has already been obtained.

A unique observation regarding the enzyme reported here is that it appears to hydrolyze all four stereoisomers of soman at about the same initial rate. Due to the presence of two centers of asymmetry in the pinacolyl moiety and the phosphorus atom, four stereoisomers (see below) of soman can be distinguished.



1. C(−) P(−)
2. C(−) P(+)
3. C(+) P(−)
4. C(+) P(+)

These isomers are assigned the symbols C(−)P(−), C(−)P(+), C(+)P(−), and C(+)P(+). The C represents the asymmetric carbon in the pinacolyl moiety and the P represents the phosphorus atom. The (−) and (+) signs refer to the relative orientation in space about the respective center of asymmetry. As shown previously, the isomers of soman vary widely in their cholinesterase inhibition rates [5, 30] and in their rate of detoxification [6, 8, 9]. It has been shown that the P(−) isomers have a high anticholinesterase activity and are therefore the most toxic, whereas the P(+) isomers are relatively nontoxic [5]. Others have shown that the less toxic, P(+), isomers appear to be preferentially degraded by enzymes from other sources that are similar to the one described here [6, 8, 9]. It is noteworthy that the liver enzyme that we have described hydrolyzed each isomer at about the same rate (Fig. 4). The liver enzyme-catalyzed hydrolysis of each stereoisomer of soman was followed as a function of time by determining the concentration of each isomer by gas chromatographic analysis. The method was tested by reproducing published results for the relative rates of inhibition of α-chymotrypsin by each of the stereoisomers of soman [13]. In the present study, to ensure reproducibility, the results shown in Fig. 4 are the average of two separate determinations (different days) using the same enzyme preparation. As can be seen, all four stereoisomers were hydrolyzed at the same rate for between one and two half-lives. The resolution of the C(+)P(−) and C(+)P(+) isomers was compromised because the difference in retention times of these two isomers is very small and close to the resolution limits of our integrator; hence C(+)P(−) values may include C(+)P(+) values at 8 and 15 min (Fig. 4B), resulting in the abnormally large value for the C(+)P(−) isomers at these two time points. The gas chromatographic evidence, which indicates that this enzyme hydrolyzes the toxic isomers of soman (Fig. 4), was confirmed by a bioassay. When soman was partially hydrolyzed (50%), its *in vivo* toxicity in mice was reduced (Table 3).

This result is particularly interesting since both whole liver homogenates [6] and rat serum [6] are highly stereospecific for the P(+) soman isomers, an observation that we were also able to confirm (data not shown). At the same time, it has been difficult to explain why both toxic and non-toxic isomers are rapidly destroyed *in vivo* [31] or upon perfusion through rat liver [32] if the hydrolytic enzymes are so highly stereospecific. Our finding of a liver hydrolase capable of catalyzing the hydrolysis of all four soman stereoisomers helps to resolve this previous discrepancy. While the serum hydrolase may be a completely separate enzyme, it should be noted that 85% of the hydrolytic activity of the liver homogenate was accounted for in the soluble fraction (Table 1). Since the enzyme did not require solubilization procedures, it is unlikely (but not impossible) that slight changes in the conformation of the enzyme occurred during isolation that modified its specificity. It is also unlikely that our soluble liver enzyme preparation was contaminated by other organophosphorus-hydrolyzing enzymes. If it had been, we would have expected very different kinetic results. Our rate values are almost identical for the hydrolysis of all of the soman stereoisomers (Fig. 4). Other published hydrolysis rate values [30] differ by 200 to 2000-fold for the (P−) versus (P+) soman stereoisomers. We feel that any contamination would have been very evident, i.e. greater than 1% would make for a 10-fold difference in our observed hydrolysis rates. Since our method reproduces prior hydrolysis data using whole liver homogenates, we feel confident that our results are real and that the differences in the data in Fig. 4B are due to instrument resolution problems.

In summary, the enzyme described here has several desirable characteristics: (1) it was localized in the soluble fraction, (2) it appeared to be a single enzyme, and (3) it hydrolyzed sarin, tabun, and soman, including the toxic soman isomers, at a significant rate. These unique properties make this enzyme an excellent candidate to be purified, partially sequenced, and its gene cloned to produce sufficient quantities of enzyme for practical application.

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