# Stereochemical Specificity of Organophosphorus Acid Anhydrolase toward *p*-Nitrophenyl Analogs of Soman and Sarin

Craig M. Hill,* Wen-Shan Li,* Tu-Chen Cheng,† Joseph J. DeFrank,†
and Frank M. Raushel*,[1]

*\*Department of Chemistry, Texas A&M University, P.O. Box 30021, College Station,
Texas 77842-3021; and †Environmental Technology Team, U.S. Army Edgewood Research,
Development and Engineering Center, Aberdeen, Maryland 21010*

Organophosphorus acid anhydrolase (OPAA) catalyzes the hydrolysis of *p*-nitrophenyl analogs of the organophosphonate nerve agents, sarin and soman. The enzyme is stereoselective toward the chiral phosphorus center by displaying a preference for the $R_P$-configuration of these analogs. OPAA also exhibits an additional preference for the stereochemical configuration at the chiral carbon center of the soman analog. The preferred configuration of the chiral carbon center is dependent upon the configuration at the phosphorus center. The enzyme displays a two- to four-fold preference for the $R_P$-enantiomer of the sarin analog. The $k_{cat}/K_m$ of the $R_P$-enantiomer is 250 $M^{-1}$ $s^{-1}$, while that of the $S_P$-enantiomer is 110 $M^{-1}$ $s^{-1}$. The order of preference for the stereoisomers of the soman analog is $R_P S_C > R_P R_C > S_P R_C > S_P S_C$. The $k_{cat}/K_m$ values are 36,300 $M^{-1}$ $s^{-1}$, 1250 $M^{-1}$ $s^{-1}$, 80 $M^{-1}$ $s^{-1}$ and 5 $M^{-1}$ $s^{-1}$, respectively. The $R_P S_C$-isomer of the soman analog is therefore preferred by a factor of 7000 over the $S_P S_C$-isomer.  © 2001 Academic Press

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## INTRODUCTION

Organophosphorus acid anhydrolase (OPAA) from *Alteromonas* sp. JD6.5 is one member of an expanding class of enzymes that is capable of catalytically hydrolyzing a wide variety of organophosphorus compounds. DNA sequence and biochemical evidence have indicated that OPAA belongs to a proline dipeptidase family of enzymes (EC 3.4.13.9) *(1,2)*. Enzymes of this type catalyze the cleavage of the peptide bond within a dipeptide containing a prolyl residue at the carboxy terminus (Xaa-Pro). Organophosphorus substrates of OPAA include the insecticide paraoxon (*O, O*-diethyl

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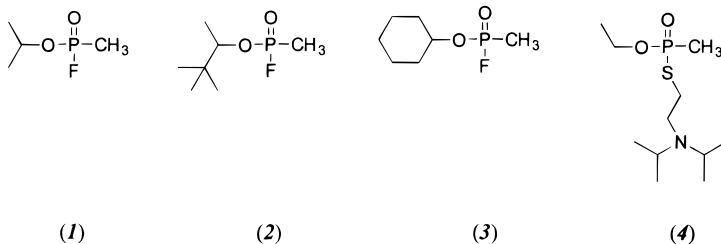
**SCHEME 1.** The phosphonofluoridates sarin (**1**), soman (**2**), GF (**3**), and the phosphonothiolate VX (**4**).

*p*-nitrophenyl phosphate) and the phosphonofluoridate chemical warfare (CW) agents sarin (**1**), soman (**2**) and GF (**3**) (*3,4*). However, the V-class agent VX (**4**) is not a substrate for this enzyme. All of these organophosphorus compounds have a chiral phosphorus center while soman has an additional stereogenic carbon center within the *O*-pinacolyl substituent (Scheme 1). The reaction catalyzed by this enzyme with the insecticide paraoxon (**5**) is shown in Scheme 2.

The organophosphorus compounds are potent neurotoxins and they exert their effect by inactivation of acetylcholinesterase (AChE), an enzyme essential for repolarization of the postsynaptic neuronal membrane in the central nervous system (*5*). The sensitivity of AChE to inactivation by individual stereoisomers of sarin and soman can vary over a wide range (*6*). It has been demonstrated that the $S_P$-stereoisomers of both sarin and soman are significantly more toxic than the $R_P$-stereoisomers of these compounds (*6*). For example, the $S_P$-enantiomer of sarin inactivates bovine erythrocyte AChE with a second-order rate constant of $1.4 \times 10^7$ M$^{-1}$ min$^{-1}$, while the rate constant for the $R_P$-enantiomer is $3 \times 10^3$ M$^{-1}$ min$^{-1}$. Similarly, inactivation of electric eel AChE by the two $S_P$-stereoisomers of soman, is characterized by rate constants of $1.8–2.8 \times 10^8$ M$^{-1}$ min$^{-1}$, while the rate constants for the $R_P$-stereoisomers are $< 3 \times 10^5$ M$^{-1}$ min$^{-1}$ (*6*). Thus, the $S_P$-stereoisomers of sarin and soman inactivate AChE $10^3$- to $10^4$-fold faster than those of the $R_P$-configuration. The preference of AChE for soman of the $S_P$-configuration is reflected in the relative lethality of the individual stereoisomers. The two $S_P$-stereoisomers have LD$_{50}$ values of 38–99 $\mu$g/kg for mice while the two $R_P$-stereoisomers have values in excess of 2000 $\mu$g/kg (*6*). Scheme 3 illustrates the structures of the six stereoisomers of sarin and soman.

OPAA has reported $k_{cat}$ values of 2500 s$^{-1}$ and 380 s$^{-1}$ for the racemic mixtures
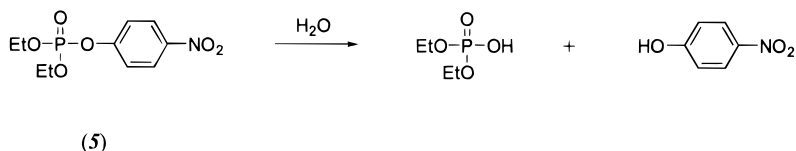


**SCHEME 2.** Hydrolysis of paraoxon (**5**) by OPAA.
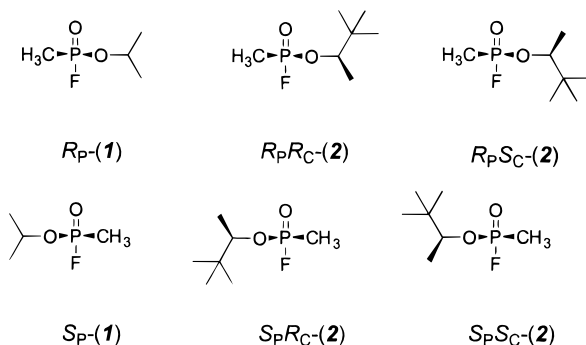
**SCHEME 3.**  The individual stereoisomers of sarin (**1**) and soman (**2**).

of soman and sarin, respectively (*7*). The enzyme has been proposed as an enzymatic alternative for the catalytic detoxification of G-agent neurotoxins (*7*). Therefore, it is of critical importance to determine the stereospecificity of this enzyme toward such neurotoxins. The substrate and stereochemical specificity of OPAA toward *p*-nitrophenyl phosphotriesters has been previously characterized with 16 paraoxon analogs (*8*). The enzyme exhibits stereoselectivity toward these substrates with a clear preference for the $S_P$-enantiomer over the $R_P$-enantiomer. OPAA displays the greatest discrimination toward the methyl ethyl and methyl isopropyl *p*-nitrophenyl phosphate substrates, where 112- and 100-fold preferences for the $S_P$-enantiomers were observed, respectively. The chiral selectivity was reduced to a 14-fold preference for the $S_P$-enantiomer of methyl phenyl *p*-nitrophenyl phosphate and the stereoselectivity is almost eliminated when the methyl group is replaced with other substituents.

In this report we investigated the stereoselectivity of OPAA toward *p*-nitrophenyl phosphonate analogs of sarin and soman. The stereoselective properties of this enzyme were characterized with racemic mixtures and preparations of individual enantiomers. The results clearly indicate that OPAA will have a stereoselective preference for the $R_P$-enantiomers of sarin and soman.

## MATERIALS AND METHODS

### *Chemical Syntheses*

Racemic mixtures of *O*-isopropyl *p*-nitrophenyl methylphosphonate (**6**) and *O*-pinacolyl *p*-nitrophenyl methylphosphonate (**7**) were synthesized by modification of standard procedures (*9–11*) (Scheme 4). Individual enantiomers of **6** were isolated through a kinetic resolution of the racemic mixture using an enzymatic method employing wild-type and mutant variants of the phosphotriesterase (PTE) from *Pseudomonas diminuta* (*12*). The enantiomeric purity of the two sarin analogs was verified by chiral capillary electrophoresis (*13*). For the preparation of $S_P$-**6,** the ratio of $S_P$-**6** to $R_P$-**6** was found to be 99:1. For the preparation of $R_P$-**6,** the ratio of $R_P$-**6** to $S_P$-**6** was found to be 93:7. The diastereomeric mixture of **7** containing the $R_P S_C$- and $S_P S_C$-stereoisomers was prepared by chemical resolution of racemic pinacolyl alcohol
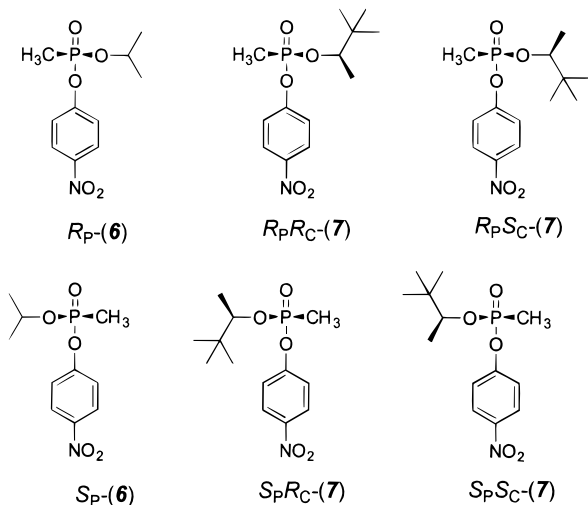
**SCHEME 4.**   The individual *p*-nitrophenyl analogs of sarin (**6**) and soman (**7**).

prior to coupling with bis(*p*-nitrophenyl)methylphosphonate (*6,14*). The individual isomers were enzymatically resolved from the diastereomeric mixture using PTE (*12*). The $R_PR_C/S_PR_C$ diastereomeric mixture of **7** and the individual isomers were prepared in a similar manner. The purity of the single stereoisomer preparations were verified by chromatography using a chiral HPLC column ((*R, R*)-Whelk-O,1) from Regis Technologies. The stereochemical purity for the individual preparations of the $R_PR_C$-, $R_PS_C$-, $S_PR_C$-, and $S_PS_C$-stereoisomers of the soman analog **7** was 90, 92, 92, and 98%, respectively. In addition, a totally racemic mixture of **7** was prepared. The synthetic and enzymatic procedures yield three different preparations of **6** and seven different preparations of **7.** The toxicity of these compounds has not been determined and thus they should be used with caution.

## Purification of OPAA

An *Escherichia coli* XL1 culture containing the plasmid pTCJS4 was grown at 37°C in 5 liters of LB containing 0.1 mM $MnCl_2$. Protein expression was induced by the addition of 0.6 mM IPTG to the cell culture at $A_{600} = 0.5$. Incubation was continued at 37°C for another 5 h. The cells were harvested by centrifugation and disrupted by two passages through a French pressure cell. Cell debris was removed by centrifugation and the supernatant solution fractionated with $(NH_4)_2SO_4$ at 40–65% saturation. The pellet was dissolved in 10 mM Bis-Tris Propane (pH 7.2) containing 0.1 mM $MnCl_2$ (buffer A) and dialyzed against the same buffer. The protein solution was applied to a Q-Sepharose column (3 × 14.5 cm) and loosely bound material was removed by washing the column with buffer A containing 0.2 M NaCl. The enzyme was eluted from the column with a linear gradient of buffer A containing 0.2–0.6 M NaCl. The enzyme eluted at ~350 mM NaCl. Fractions containing the enzyme were