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Mutant Acetylcholinesterases as Potential Detoxification Agents for Organophosphate Poisoning*

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ABSTRACT. It has been demonstrated that cholinesterases (ChEs) are an effective mode of pretreatment to prevent organophosphate (OP) toxicity in mice and rhesus monkeys. The efficacy of ChE as a bioscavenger of OP can be enhanced by combining enzyme pretreatment with oxime reactivation, since the scavenging capacity extends beyond a stoichiometric ratio of ChE to OP. Aging has proven to be a major barrier to achieving oxime reactivation of acetylcholinesterase (AChE) inhibited by the more potent OPs. To further increase the stoichiometry of OP to ChE required, we have sought AChE mutants that are more easily reactivated than wild-type enzyme. Substitution of glutamine for glutamate (E_{199}) located at the amino-terminal to the active-site serine (S_{200}) in *Torpedo* AChE generated an enzyme largely resistant to aging. Here we report the effect of the corresponding mutation on the rate of inhibition, reactivation by 1-(2-hydroxyiminomethyl-1-pyridinium)-1-(4-carboxyaminopyridinium)-dimethyl ether hydrochloride (HI-6), and aging of mouse AChE inhibited by C(+)P(−)- and C(−)P(−)-epimers of soman. The E_{202} to Q mutation decreased the affinity of soman for AChE, slowed the reactivation of soman-inhibited AChE by HI-6, and decreased the aging of mutant AChE. These effects were more pronounced with C(−)P(−)-soman than with C(+)P(−)-soman. *In vitro* detoxification of soman and sarin by wild-type and E_{202}Q AChE in the presence of 2 mM HI-6 showed that, E_{202}Q AChE was 2–3 times more effective in detoxifying soman and sarin than wild-type AChE. These studies show that these recombinant DNA-derived AChEs are a great improvement over wild-type AChE as bioscavengers. They can be used to develop effective methods for the safe disposal of stored OP nerve agents and potential candidates for pre- or post-exposure treatment for OP toxicity. BIOCHEM PHARMACOL 54;2:269–274, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. organophosphate; acetylcholinesterase inhibition; aging; stereoisomers of soman; reactivation; bioscavenger

OP** reacts with AChE (EC 3.1.1.7) and BChE (EC 3.1.1.8) to produce phosphylated enzyme conjugates with the active-center serine (S_{200}) [1, 2]. These conjugates, while relatively slow in turnover, have two dominant modes of reaction [2–7]. Spontaneous reactivation results from cleavage of the serine—O—P bond and results in the regeneration of active-site serine. ChEs inhibited by OPs can also be reactivated by various oxime nucleophiles such as 2-PAM and HI-6. However, they are only effective if the ChE–OP complex has not undergone a prior aging reaction [2]. The capacity of an oxime to regenerate active enzyme depends on the structure of inactivating OP, the structure of oxime, and the source of ChE [8].

Aging results from cleavage of the alkoxy—O—P bond, forming the corresponding P—O^- containing conjugates that can no longer be reactivated [2, 3, 8]. The rate of aging depends on the structure of OP, the source of enzyme, pH, temperature, and ionic strength of the solution [3, 9, 10]. Analysis of the kinetics and pH profiles of aging suggests two potential mechanisms for aging: a general acid-catalyzed reaction and one catalyzed by a nucleophile/activated H_2O. The acid or low pH-catalyzed rate of aging appears most rapid with OP–AChE conjugates containing tertiary alkoxy groups, followed by secondary and primary alkoxy groups. This suggests that a carbonium ion may serve as an intermediate [2, 4, 11].

In particular, ChEs inhibited by the nerve agent soman are refractory to reactivation by oximes because they age very rapidly [3]. Studies of the pH dependence of the dealkylation reaction of soman-inhibited AChE indicated participation of a residue with pK 4.5 and another residue with pK 6.0 [3, 10]. Based on the X-ray crystal structure of

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** *Abbreviations:* BChE, butyrylcholinesterase; ChE, cholinesterase; FBS AChE, fetal bovine serum acetylcholinesterases; HI-6, 1-(2-hydroxyiminomethyl-1-pyridinium)-1-(4-carboxyaminopyridinium)-dimethyl ether hydrochloride; OP, organophosphate; 2-PAM, 2-(hydroxyiminomethyl)-1-methylpyridinium iodide; sarin, O-isopropyl methylphosphonofluoridate; and soman, O-pinacolyl methylphosphonofluoridate.

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Torpedo AChE, these residues were proposed to be E_{199} and H_{440}. Molecular modeling and site-directed mutagenesis studies with *Torpedo* AChE suggested that electrostatic interactions between the carbonium ion and the carboxylate of E_{199} could facilitate the dealkylation reaction [12, 13].

Besides aging, steric hindrance may also be important in the reactivation reaction [13, 14]. Due to the presence of two chiral centers, one at the α-carbon atom of the pinacolyl moiety and the other at the phosphorus atom, soman is a mixture of four stereoisomers. The *in vitro* toxicologic and anticholinesterase properties of the isomers of soman are very different [15]. Of the four stereoisomers, only the two P(−)-epimers are potent inhibitors of AChE [16, 17]. *In vivo* studies with mice challenged with a dose of one LD_{50} of racemic soman showed that toxicity arose primarily from the C(−)P(−)-epimer [17]. *In vitro* inhibition and reactivation studies with C(+)P(−)- and C(−)P(−)-soman-inhibited AChE indicated that the two diastereomeric-enzyme conjugates had different rates of reactivation, which depend on the source of the enzyme [18].

Recently we showed that mutation of glutamate (E_{199}) located at the amino-terminal to the active-site serine (S_{200}) in *Torpedo* AChE generated a mutant AChE that was largely resistant to aging [12]. The loss of charge appeared to affect the intrinsic rate of aging of mutant AChE, suggesting that the negative charge on E_{199} facilitated the removal of the alkoxy group. Here we have extended these studies using mouse $E_{202(199)}Q$* AChE to determine the effect of this mutation on the rate of inhibition, reactivation by HI-6, and aging of mouse AChE inhibited by the two P(−)-epimers of soman. The *in vitro* detoxification of soman and sarin by wild-type and $E_{202}Q$ AChE in the presence of 2 mM HI-6 was also studied.

MATERIALS AND METHODS

Materials

Wild-type and $E_{202}Q$ mutant of mouse AChE were expressed, purified, and characterized with respect to catalytic parameters as described [20]. One nanomole of wild-type AChE was equivalent to 132 units, and 1 nmol of $E_{202}Q$ mutant AChE was equivalent to 60 units. Soman was obtained from the Chemical Research, Development and Engineering Center (Aberdeen Proving Ground, MD). Soman used in these experiments was 98.6% pure when analyzed by [^{31}P] nuclear magnetic resonance. The two P(−)-epimers of soman were obtained as described previously [21]. Concentrations of soman solutions were determined by titration of the solution with a known amount of FBS AChE and measurement of residual activity (1 nmol of FBS AChE is equivalent to 400 units). The oxime, HI-6, was obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Washington, DC).

Titration of AChE with Soman

Dilutions of racemic soman, or C(+)P(−)-soman, or C(−)P(−)-soman, in saline (1–10 μL), were added to wild-type or $E_{202}Q$ mutant AChE (0.5 U/mL) in 10 mM sodium phosphate, pH 8.0, containing 0.05% BSA. Samples were incubated for 30 min at room temperature and assayed for residual enzyme activity using the Ellman assay [22]. Residual AChE activity was plotted against the concentration of soman added to the reaction mixture to ascertain the stoichiometry between AChE and soman.

Inhibition of AChE with Soman

Inhibition of mouse wild-type AChE (0.1 U/mL; 0.76 nM) by soman, run in 50 mM sodium phosphate, pH 8.0, containing 0.05% BSA, was initiated by adding racemic soman (2.2 to 4.4 nM) or the two P(−)-epimers of soman (0.55 to 2.2 nM) and measuring enzyme activity at various time intervals. A similar procedure was used for $E_{202}Q$ AChE (0.1 U/mL; 1.67 nM) except that the amount of racemic soman and the two P(−)-epimers used was 4.4 to 17.6 and 2.2 to 8.8 nM, respectively. Experiments were carried out with at least four different concentrations of soman. The apparent bimolecular rate constants for the inhibition reactions measured under second-order conditions were determined by non-linear regression of the plot of $\{AChE_t/(OP_0 - [AChE_0 - AChE_t])\}$ versus time at different inhibitor concentrations [23], where $AChE_t$ is the enzyme concentration at time t, $AChE_0$ is the initial enzyme concentration at time t = 0, and OP_0 is the initial concentration of OP.

Reactivation of Soman-Inhibited AChE with HI-6

Mouse wild-type (8.0 U/mL) and $E_{202}Q$ AChE (12 U/mL) in 50 mM Tris · HCl, pH 9.5, were inhibited with stoichiometric amounts of soman or one of its P(−)-epimers for 15 min at 4°. Reactivation was started by mixing 10 μL of the soman–AChE conjugate with 90 μL of sodium phosphate, pH 8.0, containing HI-6 at a final concentration of 2 mM at 25°. Enzyme activity of aliquots of the reactivation mixture were measured at intervals of 0.5, 1, 2, 3, 4, 6, and 24 hr using the Ellman method [22]. Data for the time-course of reactivation reactions were analyzed by nonlinear regression analysis using the equation [14]:

$$\%(AChE_{reac})_t = A[1 - e^{-kt}] \quad (1)$$

where A is the percent-amplitude of the reactivatable form of soman-inhibited AChE and k is the pseudo first-order rate constant for reactivation.

* The dual numbering system gives the residue number in the species designated followed by the corresponding residue in *Torpedo* AChE [19].

pH Profile for the Aging of AChE

Mouse wild-type (8 U/mL) and $E_{202}Q$ AChE (12 U/mL) in 50 mM Tris · HCl, pH 9.5, were inhibited with stoichiometric amounts of soman or one of its P(−)-epimers for 15 min at 4°. Fifty microliters of soman–AChE conjugate was diluted into 450 μL of one of the 50 mM buffer solutions at different pH values at 25°, and the pH of all samples was monitored using the pH meter. The following buffer solutions were used: sodium acetate at pH 5.0 and 5.5; sodium phosphate at pH 6.0, 6.5, 7.0, 7.5, and 8.0; and Tris · HCl at pH 8.5. Parallel samples without soman were used to monitor the stability of enzyme at each pH. Aliquots (40 μL) were removed at various time intervals and transferred to tubes containing 10 μL of 10 mM HI-6 in 50 mM sodium phosphate, pH 8.0. Samples were incubated overnight at room temperature before assay for AChE activity using the Ellman method [22].

In Vitro Detoxification of OPs by Mouse Wild-Type and $E_{202}Q$ Mutant AChE

The incubation mixture in 50 mM sodium phosphate, pH 8.0 (total volume 0.5 mL), contained 0.05% BSA, 0.011 nmol mouse wild-type or $E_{202}Q$ AChE, 2 mM HI-6, and 0.275 nmol soman. Tubes of buffer containing enzyme alone, enzyme with HI-6, and enzyme with soman were incubated as controls. After incubating for 30 min, 10-μL aliquots from each tube were assayed for inhibition of AChE activity. Samples (10 μL) were assayed after 6 hr to measure recovery of AChE activity. The addition of soman (final concentration of 0.275 nmol) to the tube containing soman and HI-6, and buffer to the control samples followed by measurement of AChE activity was repeated until the AChE activity decreased to 10% of its original value. The detoxification of sarin by wild-type and $E_{202}Q$ AChE was conducted using a similar procedure except that the addition of 1.8 nmol of sarin was repeated every 24 hr. Residual enzyme activity was plotted against the cumulative amount of soman or sarin present in the reaction mixture.

RESULTS AND DISCUSSION

The crystal structure of the *Torpedo* AChE shows E_{199} to reside at the base of the active-center gorge, and its van der Waals surface appears to reside within 1.5 Å of that of the quaternary ammonium group of the substrate or inhibitor bound to the active center of the enzyme [24, 25]. Molecular modeling studies of the adducts of *Torpedo* AChE with the two P(−)-epimers of soman indicated that E_{199} was located at a distance of 3.3 Å from the chiral carbon of the pinacolyl moiety. Therefore, the carboxylate side chain could participate in the stabilization of the carbonium ion in the transition state for dealkylation [13]. These models were substantiated by site-directed mutagenesis studies using *Torpedo* and human AChE [12, 23].

Figure 1 shows the titration of mouse wild-type and

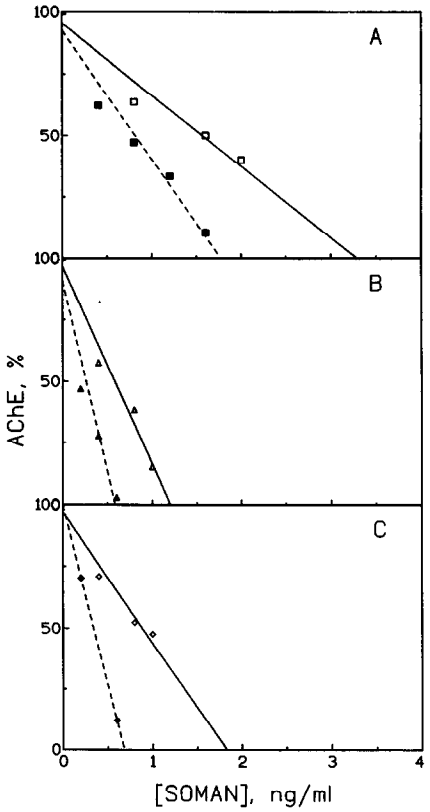

FIG. 1. Titration of mouse wild-type and $E_{202}Q$ mutant AChE with the stereoisomers of soman. Increasing amounts of racemic soman (A), or C(+)P(−)-soman (B), or C(−)P(−)-soman (C), in 1- to 10-μL aliquots, were added to wild-type (0.5 U/mL; 3.8 nM) or $E_{202}Q$ mutant AChE (0.5 U/mL; 8.35 nM) in 50 mM sodium phosphate, pH 8.0. The final concentrations of soman were 2–20 nM. Samples were incubated for 30 min and assayed for residual enzyme activity using the Ellman assay [22]. Data for wild-type (---) and $E_{202}Q$ (—) AChE were plotted as percent of residual AChE activity at various concentrations of soman. The data shown are representative of three experiments.

$E_{202}Q$ AChE with racemic soman (panel A) and its two P(−)-epimers (panels B and C). At the concentrations of soman employed, the approach to inhibition appeared practically stoichiometric for wild-type and mutant AChE. Differences in the titration curves for wild-type and $E_{202}Q$ AChE with racemic soman, C(+)P(−)-soman, and C(−)P(−)-soman reflect the 2-fold difference in the k_{cat} for acetylthiocholine hydrolysis between wild-type and mutant enzymes.

To examine the role of $E_{202(199)}$ in the stereoselectivity of AChE for the two P(−)-epimers of soman, we compared the bimolecular rate constants for the inhibition of wild-type and $E_{202}Q$ AChE by racemic soman and the two P(−)-epimers of soman (Table 1). Racemic soman is a mixture of four stereoisomers of which only the two P(−)-epimers are potent inhibitors of AChE [16, 17]. Therefore, the values for the bimolecular rate constants for wild-type and mutant AChE with racemic soman should be effectively twice that reported in Table 1. A 2-fold difference in the bimolecular rate constant of wild-type AChE

TABLE 1. Bimolecular rate constants for the inhibition of mouse acetylcholinesterase by stereoisomers of soman

Inhibitor	$k \times 10^{-8}$ (M^{-1} min^{-1})	
	Wild-type	$E_{202}Q$
Racemic soman	0.48 ± 0.06	0.048 ± 0.002
C(+)P(−)-Soman	1.20 ± 0.27	0.200 ± 0.017
C(−)P(−)-Soman	0.69 ± 0.09	0.044 ± 0.004

Values for the bimolecular rate constants of inhibition, k, were measured under second-order reaction conditions as described in Materials and Methods. Values are the means ± SD from three experiments.

for the two P(−)-epimers was observed compared with a 4.5-fold difference observed for $E_{202}Q$ AChE. Previous inhibition studies with stereoisomers of soman also showed that the C(+)P(−)-soman was a slightly more potent inhibitor than the C(−)P(−)-epimer. Bovine erythrocyte AChE exhibited a 6-fold difference in the bimolecular rate constants for the two P(−)-epimers as compared with a 1.6-fold difference observed for electric eel AChE [17, 18]. The difference in the bimolecular rate constants of wild-type and $E_{202}Q$ AChE for racemic soman, C(+)P(−)-soman, and C(−)P(−)-soman was 10-, 6-, and 16-fold, respectively. These results suggest that the loss of charge on E_{202} decreased the reactivity of soman for mutant AChE and the reactivity of C(−)P(−)-soman was affected more than that of the C(+)P(−)-epimer.

The influence of E_{202} to Q mutation on HI-6-induced reactivation of wild-type and $E_{202}Q$ AChE inhibited by the two P(−)-epimers of soman was also studied. For AChE inhibited with racemic soman or either of the two P(−)-epimers, the rate of reactivation of wild-type AChE was 7- to 8-fold faster than that of $E_{202}Q$ AChE (Table 2 and Fig. 2A–C). For both enzymes, C(−)P(−)-inhibited AChE reactivated at about half the rate of C(+)P(−)-inhibited AChE. Similar studies with racemic 7-(methylethoxyphosphinyloxy)-1-methylquinolinium iodide (MEPQ)-inhibited mouse AChE showed that the mutation of E_{202} to Q resulted in a 16- to 33-fold reduction in the rate of reactivation of the enzyme by 2-PAM and HI-6 [14]. Reactivation of wild-type AChE inhibited with either epimer of soman was rapid and virtually complete (Fig. 2, panels B and C), as compared with $E_{202}Q$ AChE, which could be reactivated to about 70% from the AChE-C(−)P(−)soman conjugate (Fig. 2C). The mutation affects the rate of reactivation as well as the extent of reactivation especially for C(−)P(−)-soman-AChE. These results are consistent with molecular modeling studies, which showed that steric hindrance between the methyl group at the chiral carbon of C(−)P(−)-soman and H_{440} can reduce the efficiency of nucleophilic reactivation of C(−)P(−)-soman-AChE compared with C(+)P(−)-soman-AChE [13]. The results are also consistent with *in vitro* studies with human, electric eel, and plaice AChE, which demonstrated that reactivation of C(+)P(−)-soman-AChE by HI-6 was more effective than the reactivation of C(−)P(−)-soman-AChE [17, 18, 26, 27].

TABLE 2. Rate constants for the reactivation of soman-inhibited mouse acetylcholinesterase by 2 mM HI-6

Inhibitor	k (min^{-1})	
	Wild-type	$E_{202}Q$
Racemic soman	0.07 ± 0.02	0.01 ± 0.002
C(+)P(−)-Soman	0.08 ± 0.01	0.01 ± 0.002
C(−)P(−)-Soman	0.04 ± 0.01	0.006 ± 0.001

Values for the rate constants for the reactivation of soman-inhibited AChE, k, were obtained by analyzing the data for the time course of the reactivation reactions using equation 1 [14]. Values are the means ± SD from three experiments.

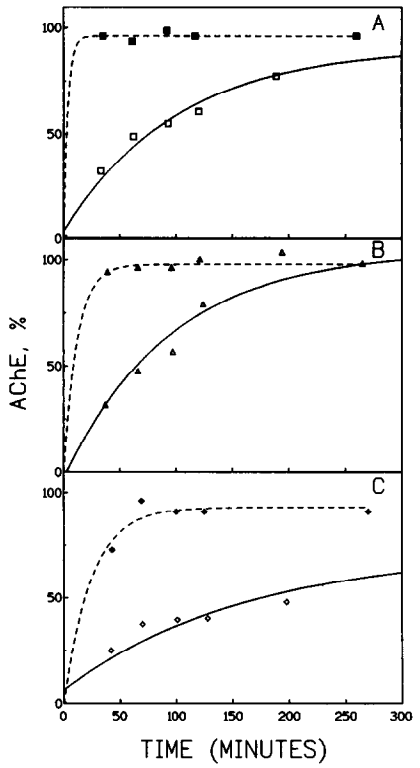

FIG. 2. Time–course for the reactivation of soman-inhibited mouse wild-type and $E_{202}Q$ mutant AChE with 2 mM HI-6. Mouse wild-type and $E_{202}Q$ AChE (60 nM; 10 U/mL) in 50 mM Tris · HCl, pH 9.5, were inhibited with stoichiometric amounts of racemic soman (A) or C(+)P(−)-soman (B) or C(−)P(−)-soman (C). Reactivation was started by mixing 10 μL of the soman–AChE conjugate with 90 μL of sodium phosphate, pH 8.0, containing HI-6 at a final concentration of 2 mM. Enzyme activity of aliquots of the reactivation mixture were measured at intervals of 0.5, 1, 2, 3, 4, 6, and 24 hr using the Ellman method [22]. Data for wild-type (---) and $E_{202}Q$ (—) AChE were plotted as percent of AChE control activity without soman. The data shown are representative of three experiments.

A reaction that counteracts reactivation of soman-inhibited AChE is aging. We examined the pH dependence of aging of wild-type and $E_{202}Q$ mutant AChE inhibited by soman and the two P(−)-epimers by their incubation at various pHs and subsequent measurement of the extent of reactivation by 2 mM HI-6 at pH 8.0. Unlike *Torpedo* $E_{199}Q$ AChE and human $E_{202}Q$ AChE, the rate constants

TABLE 3. pH Dependence of the rate constants for the aging of mouse wild-type and $E_{202}Q$ mutant acetylcholinesterases

	k (min^{-1})*					
	Racemic soman		C(+)P(−)-Soman		C(−)P(−)-Soman	
pH	Wild-type	$E_{202}Q$	Wild-type	$E_{202}Q$	Wild-type	$E_{202}Q$
5.0	0.39 ± 0.04†	0.11 ± 0.02†	0.27 ± 0.04†	0.066 ± 0.01	0.35 ± 0.05†	0.04 ± 0.01
6.2	0.23 ± 0.01†	0.04 ± 0.01	0.33 ± 0.05†	0.035 ± 0.01	0.27 ± 0.04†	0.02 ± 0.002
6.5	0.19 ± 0.02†	0.05 ± 0.01	0.19 ± 0.01†	0.025 ± 0.005	0.25 ± 0.04†	0.01 ± 0.003
6.8	0.21 ± 0.03†	0.04 ± 0.014	0.21 ± 0.03†	0.024 ± 0.007	0.19 ± 0.02†	0.022 ± 0.016
7.3	0.07 ± 0.01	0.003 ± 0.001	0.066 ± 0.01	0.006 ± 0.001	0.09 ± 0.01	0.003 ± 0.001
7.9	0.023 ± 0.005	0.003 ± 0.001	0.036 ± 0.01	0.003 ± 0.001	0.05 ± 0.01	ND‡
8.5	0.03 ± 0.008	ND	0.02 ± 0.006	ND	0.04 ± 0.01	ND

* Determined by non-linear regression analyses of kinetic data. Values are averages ± the range from two experiments.
† Estimates from the residual portion of the kinetic profiles.
‡ ND: Aging not detected over a 24-hr period.

for the aging of mouse $E_{202}Q$ mutant AChE were only 8- to 24-fold lower at pH 7.0 to 7.5 than wild-type AChE [12, 23]. At pH values below 7.3, aging of the wild-type enzyme was too rapid to measure the aging constants accurately. No differences in the rate constants for the aging of wild-type AChE inhibited with C(+)P(−)- and C(−)P(−)-soman were observed. These results are consistent with previous observations made with human, eel, and bovine erythrocyte AChE, which showed that the rate of aging was independent of the configuration at the α-carbon atom of the pinacolyl moiety of soman [17, 27]. For $E_{202}Q$ AChE, at pH 8.0 and above, the rate of aging was so slow that it could not be measured under present experimental conditions (Table 3). At pH values below 8.0, the rate of aging of C(+)P(−)-soman-$E_{202}Q$ AChE was two times faster than the rate of aging of C(−)P(−)-soman-$E_{202}Q$ AChE. Wild-type and $E_{202}Q$ AChE inhibited with soman or the P(−)-epimers showed an increase in the rate of aging with decreasing pH, consistent with an aging mechanism involving a carbonium-ion intermediate. A similar pH dependence for the soman-*Electrophorus* AChE and soman-*Torpedo* AChE conjugate was observed previously [3, 12]. The pH profile for the aging of soman-inhibited wild-type mouse AChE between pH 5.0 and 8.5 indicated the participation of a residue with an estimated pK ~6.4, which is in agreement with a previously reported value of 6.4 for electric eel AChE [3] and 6.0 for bovine erythrocyte AChE [10].

Aging has proven to be the major barrier to achieving oxime reactivation of AChE inhibited by the more potent OPs [28]. Recombinant enzymes without this liability would confer a superior characteristic in the development of catalytic scavengers of OPs. To test this possibility, we carried out *in vitro* detoxification of sarin and soman by wild-type and $E_{202}Q$ AChE in the presence of 2 mM HI-6 as shown in Figure 3. In the presence of 2 mM HI-6 (the extrapolated sarin/AChE ratio was 2800 for wild-type AChE and 7200 for $E_{202}Q$ AChE), the same amount of wild-type and $E_{202}Q$ AChE could neutralize 2800- and 7200-fold molar excess of sarin, respectively (Fig. 3A). Similarly, the extrapolated soman/AChE ratio was 135 for wild-type AChE and 225 for $E_{202}Q$ AChE, suggesting that in the presence of 2 mM HI-6, the same amount of wild-type and $E_{202}Q$ AChE could detoxify 135- and 225-fold molar excess of soman, respectively (Fig. 3B). The overall effect of the reduced reactivity of $E_{202}Q$ AChE with soman and reduced rates for the reactivation and aging of soman-inhibited $E_{202}Q$ AChE is a 2- to 3-fold increase in detoxification of soman and sarin compared with wild-type

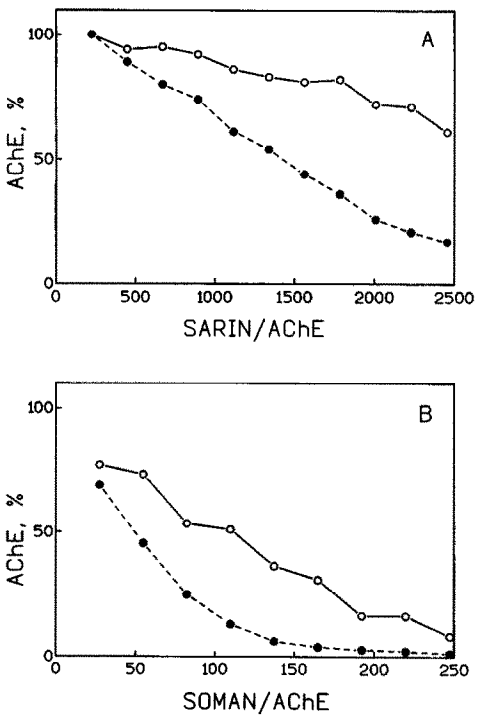

FIG. 3. *In vitro* detoxification of sarin or soman by mouse wild-type and $E_{202}Q$ mutant AChE in the presence of HI-6. The reactivation of mouse wild-type (●) and $E_{202}Q$ (○) AChE (0.011 nmol) was carried out in the presence of 50 mM sodium phosphate, pH 8.0, containing 0.05% BSA and 2 mM HI-6 after repeated additions of sarin (1.8 nmol, panel A) at 24-hr intervals, or soman (0.275 nmol, panel B) at 6-hr intervals. Residual enzyme activity was plotted against the cumulative amount of soman or sarin present in the reaction mixture. The data shown are representative of three experiments.

AChE. These results suggest that such recombinant DNA-derived AChEs, in which the glutamate located at the amino-terminal to the active-site serine is substituted by glutamine, are a great improvement over wild-type AChE as bioscavengers. These recombinant enzymes can be used to develop effective methods for the safe disposal of stored OP nerve agents and appropriate formulations for medical, surgical, and skin decontaminants and also for decontamination of materials, equipment, and the environment. The major requirements for an enzyme to be an effective bioscavenger for OP toxicity are: (a) relatively long half-life in circulation, (b) relatively high turnover number, (c) immunocompatibility, and (d) availability in sufficient quantities for use as a pretreatment drug. These recombinant DNA-derived ChEs have to fulfill these criteria before they can be tested as bioscavengers.

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