Biochemical Pharmacology, Vol. 36, No. 21, pp. 3579–3585, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

STEREOSELECTIVE HYDROLYSIS OF SOMAN IN HUMAN PLASMA AND SERUM

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(*Received* 3 *February* 1987; *accepted* 27 *April* 1987)

Abstract—The contribution of various human serum and plasma fractions to the total hydrolysis rate constants of the four isomers of soman is studied. Spontaneous hydrolysis (as measured in buffer) occurs at a faster rate for the C(+)P(+)- and C(−)P(−)-isomers. A stereoselectively catalyzed hydrolyis of soman occurs in serum fractions IV and V (albumin). In fraction V the C(+)P(+)- and C(−)P(−)-isomers are hydrolyzed at a faster rate than their respective epimers, while in fraction IV-1 a stereoselective effect towards C(+)P(+)-soman is found. All the forementioned contributions, however, are negligible in comparison with the stereoselective enzymatic hydrolysis of the P(+)-isomers. The latter reaction is characterized by a significant lowering of the activation energy as compared with the spontaneous hydrolysis of the P(+)-isomers. Such a lowering in activation energy is not found for the hydrolysis of the P(−)-isomers in whole serum or plasma; hence it can be concluded that a phosphorylphosphatase hydrolyzes the P(+)-isomers in a stereoselective way, the P(−)-isomers either not being affected by this (these) enzyme(s) or the mechanism of catalysis being fundamentally different. This conclusion is in agreement with the observations on the influence of Hg^{2+} on the hydrolysis of soman in serum; the hydrolysis of the P(+)-isomers is significantly inhibited by 1 mM of Hg^{2+} while the P(−)-hydrolysis is unaffected by this concentration of Hg^{2+}. The action of some potential inhibitors on this phosphorylphosphatase activity was studied. Iodoacetate did not inhibit nor did Ba^{2+}, Sr^{2+}, Co^{2+} or Mn^{2+} show a significant effect on the hydrolysis of the P(+)-isomers. On the other hand the hydrolytic activity in serum was nearly completely inhibited by EDTA but restored upon addition of Ca^{2+}. These findings suggest that this enzymatic activity can be classified as an arylesterase (paraoxonase). Finally, the influence of pH on the hydrolytic activity shows a different pattern for C(+)P(+)- and C(−)P(+)-soman, which may suggest that more than one enzyme is involved in the degradation of soman.

In a previous paper [1] it was shown that the degradation of soman (1,2,2-trimethylpropyl methylphosphonofluoridate) in human serum involves hydrolysis as well as irreversible binding to proteins.

In biological fluids, such as serum, spontaneous hydrolysis is part of the expected hydrolysis processes, and may be catalyzed by ions or molecules. Recently Benschop *et al.* [2] described a catalytic effect of phosphate ions on the hydrolysis of soman in rat serum.

Enzymatic activity for the hydrolysis of ester bonds does exist in biological tissues. This esteratic activity can generally be attributed to a particular class of enzymes, the ester-hydrolases or esterases. Organophosphorus anti-cholinesterase agents are hydrolyzed by a subcategory of esterases, the A-esterases [3]. A variety of A-esterases seems to exist. These enzymes were sometimes named after the substrate used in the respective studies, e.g. sarinase, tabunase, DFP-ase. Augustinsson and Heimbürger [4] termed the enzymes hydrolyzing organophosphorus compounds phosphorylphosphatases. Mounter [5] classified mammalian phosphorylphosphatases in two categories, specific esterases (tissue-bound enzymes) that are generally activated by Mn^{2+} and Co^{2+}, and non-specific (serum) esterases that are inhibited by Mn^{2+} and Co^{2+}, but activated by Sr^{2+} and Ba^{2+}.

The first report of an A-esterase in serum (of the rabbit) was made by Mazur [6] with DFP as a substrate. In human serum or plasma paraoxon [7], tabun [4], DFP [8], sarin [9], and several *p*-nitrophenyl phosphonates [10–12] were shown to be hydrolyzed enzymatically. Studies with mixed substrates, heat inactivation and pH-dependency [13] indicated the existence of at least three types of serum A-esterases with some overlapping specificity. Efforts were made to attribute specific activities to various serum fractions. Paraoxonase activity appeared to be localized mainly in serum fraction IV-1 [14] while fraction V (albumin) hydrolyzed preferentially ethyl *p*-nitrophenyl ethylphosphonate (armine) [11]. Fraction VI-2 of bovine plasma was reported to contain high sarinase activity [15].

Organophosphorus nerve agents generally display an asymmetric centre at phosphorus and it can be expected that esterolytic activity will also be stereoselective. A stereoselective effect towards tabun was reported by Augustinsson [16] for hog kidney phosphorylphosphatase, but not for human plasma phosphorylphosphatase. The stereospecific nature of the hydrolysis of sarin in the plasma of various species, including man, was demonstrated by Christen *et al.* [17]. It was shown that the P(+)-isomer of sarin is much more rapidly hydrolyzed than the P(−)-isomer [18]. The stereoselective enzymatic hydrolysis

of soman in rat plasma was demonstrated [9] and later, it was shown that the P(+)-isomers were rapidly hydrolyzed [19] and also that a difference in hydrolysis rate existed between the two P(+)-isomers [20]. Quantitative aspects of the differences in hydrolysis between the four stereoisomers in human serum were reported using C(±)P(±)-soman, i.e. soman containing all four stereoisomers [1]. The differentiation between the isomers was based on a separation of diastereoisomers and on a kinetic analysis of the stereoselective enzymatic hydrolysis.

In this paper the hydrolysis rate constant of each soman isomer in human serum is determined directly by using soman that is resolved in the 1,2,2-trimethylpropylmoiety, i.e. C(+)P(±)- and C(−)P(±)-soman. The relative importance of enzymatic hydrolysis by somanase was investigated and the kinetics of the hydrolysis processes in human serum or plasma, in various serum fractions and in buffer solutions was studied. More particular effects of temperature and pH were investigated and the influence of some divalent metal ions and other candidate inhibitors was determined.

MATERIALS AND METHODS

C(+)P(±)- and C(−)P(±)-soman were obtained from the Prins Maurits Laboratory, TNO, The Netherlands, with a purity >98.5% for C(+)P(±) and >99.2% for C(−)P(±); optical rotation (α_{578}^{25}) was +14.4° C(+)P(±)- and −14.8° for C(−)P(±)-soman. Neat C(±)P(±)-soman was synthesized in house according to the original synthesis of sarin by Dr G. Schrader, starting from dimethyl methylphosphonate as described by Saunders [21]. Purity was better than 95%, as measured by ^{31}P-NMR spectroscopy.

Human serum albumin (crystallized and lyophilized) and human serum Fraction IV-1 (essentially globulin free) were obtained from Sigma. Aqueous solutions of bivalent ions were obtained by dissolving the corresponding chlorides in distilled water. All inorganic chemicals and solvents were analytical grade.

Human plasma was obtained from blood sampled by venous puncture in tubes containing lithium heparin as an anticoagulant. The tubes were centrifuged and the collected supernatant was pooled. Fractions of 5–10 ml were prepared immediately and stored at −18°. Pooled human blood serum was obtained as described earlier [1].

Tris buffer was prepared with 50 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to the desired pH with a 10% NaOH or HCl solution in distilled water.

Frozen plasma or serum were allowed to thaw in a water bath at room temperature and were centrifuged to remove cryoprecipitate, if present. Where necessary dilutions were made and/or metal ions or other effectors were added. Addition was done from solutions in water (2-propanol for the organophosphorus compounds). Ten to 50 μl of solution were added per ml of plasma, serum or plasma diluate. The pH was then adjusted to 7.4 with a 10% HCl solution. During the experiments the pH did not change by more than 0.2 units.

Assay. The assay procedure was described in detail [22, 1]. On a carbowax column the diastereoisomers of soman are separated which results in two peaks. Furthermore, according to Benschop [20], when starting from C(+)P(±)-soman the peak eluting first represents the C(+)P(−)-isomer; when starting from C(−)P(±)-soman the peak eluting first represents the C(−)P(+)-isomer. When C(±)P(±) was assayed the sum of both peaks allowed the quantitation of "total" soman (sum of stereoisomers).

Degradation experiments were carried out as described previously [1].

Preincubation. Serum or plasma was spiked with C(±)P(±)-soman from a concentrated solution in 2-propanol to obtain a concentration in serum of 1 μM. The spiked serum was kept for 17 hr in a thermostated waterbath at a temperature of 25 (±0.5)°. Where appropriate, pH was readjusted to 7.4, and hydrolysis experiments were then started by adding soman to the preincubated serum. Controls revealed that no soman, from the preincubation, could be detected above a threshold of 100 pg/ml (550 pM) prior to the start of the hydrolysis experiments.

Calculation of hydrolysis rate constants. The use of partly resolved soman, i.e. C(+)P(±)- and C(−)P(±)-soman allows the direct calculation of individual hydrolysis rate constants as, in this case, a chromatographic peak corresponds to only one isomer. Calculation was done using the plot of the logarithm of the percentage remaining soman versus time using linear unweighted curve fitting. For each experiment, the 95% confidence level was determined from the estimated variance of the slope.

Calculation of activation energy. From the plot of $\ln k$ versus $1/T$ the activation energy was calculated according to the Arrhenius equation: $k = k_0 \exp(-E_a/RT)$, where k is the rate constant, k_0, the preexponential factor, R, the universal gas constant, and T, the absolute temperature. Linear unweighted curve fitting was used to calculate E_a and the 95% confidence intervals were determined from the estimated variance of the slope.

RESULTS

Spontaneous hydrolysis

It is shown (Fig. 1) that hydrolysis of the diastereoisomers of soman in Tris buffer occurs at different rates. However, at pH 7.4 the rate constants in Tris are rather small and accordingly the standard errors of the slopes become proportionally larger (Table 1). Consequently the difference between the rate constants of the diastereoisomers is only significant at a confidence interval of 85%. From Fig. 2 the values of the activation energy are calculated using C(±)P(±)-soman: 55 ± 13 kJ/mole for C(−)P(+) and C(+)P(−) and 57 ± 8 kJ/mole for C(+)P(+) and C(−)P(−). These values do not differ significantly.

Hydrolytic activity in serum fractions

Table 1 shows that the hydrolysis rates of the four isomers of soman in 0.2% fraction IV-1 in Tris do not differ from their rate constants obtained in Tris. Addition of Ca^{2+}, at concentration of 2.3 mM which is at the level usually found in serum [23], gives

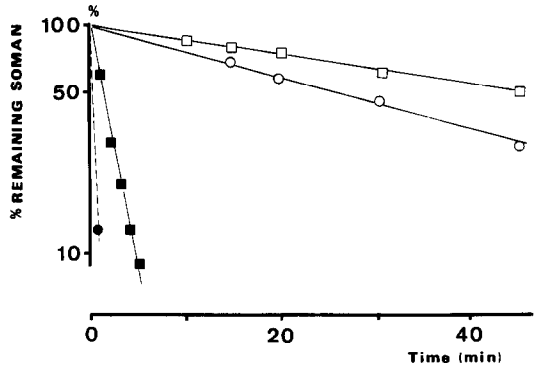

Fig. 1. Hydrolysis of soman in preincubated human plasma/Tris (50% v/v) at pH 7.4–7.6 and 37°. Soman epimers were used: C(+)P(±) 103.4 ng/ml (568 nM) and C(−)P(±), 101.3 ng/ml (556 nM). ●, C(+)P(+); □, C(+)P(−); ○, C(−)P(−); ■, C(−)P(+). Each point is the mean of at least two determinations.

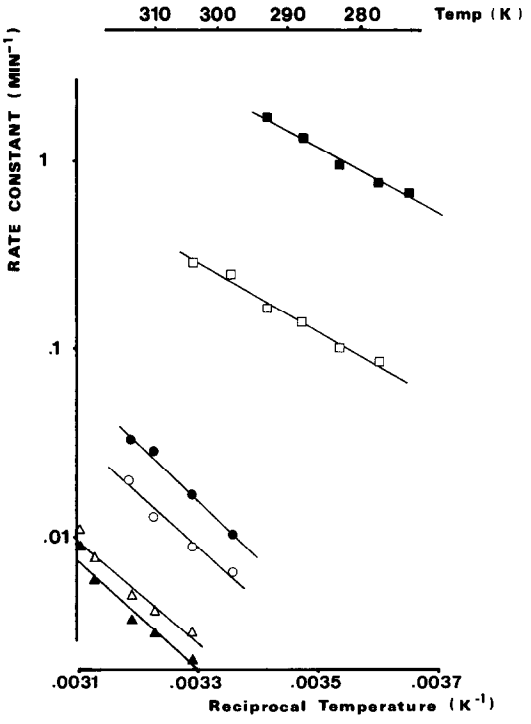

Fig. 2. Temperature dependence of hydrolysis rate constants of soman in Tris, using C(±)P(±)-soman, (△ and ▲), and in preincubated human plasma/Tris (50% v/v), using C(+)P(±)- and C(−)P(±)-soman, (○, ■, □ and ●). The pH was kept at 7.4–7.6. ■, C(+)P(+)-soman; □, C(−)P(+)-soman; ●, C(−)P(−)-soman; ○, C(+)P(−)-soman. ▲, first GLC peak containing C(−)P(+) and C(+)P(−); △, second GLC peak containing C(−)P(−) and C(+)P(+).

an enhancement of the hydrolysis, especially for C(+)P(+)soman. The activation energy for the hydrolysis of the latter isomer in fraction IV-1 was determined (Table 2). The value of 54 ± 11 kJ/mole does not differ significantly from the values obtained in Tris. In serum albumin solution a catalytic effect which distinguishes between diastereoisomers is found. Fractions I and II were also tested but no catalytic effect was detected.

Hydrolysis in plasma/Tris

From Table 1 it can be seen that in plasma, diluted with Tris, the P(+)-isomers are much more rapidly hydrolyzed than their P(−)-counterparts. As compared with the hydrolysis of P(+)isomers in solutions containing whole plasma, the hydrolysis of the P(−)-isomers in solutions containing plasma fractions IV-1 or albumin is negligible. The hydrolysis of the P(−)-isomers is of the same order of magnitude in all the solutions studied. The contributions of fraction IV-1 and albumin account for a major part of the hydrolysis of the C(+)P(−)-isomer, which is hydrolyzed more slowly than its C(−)P(−)-counterpart. For the latter isomer the contributions of fraction IV-1 and albumin in the total hydrolysis process are also substantial but proportionately smaller than for the C(+)P(−)-isomer.

An Arrhenius-plot for the hydrolysis of the four stereoisomers of soman in plasma/Tris is shown in Fig. 2. From this plot activation energies can be calculated. The values are shown in Table 2: in plasma/Tris the activation energy is significantly lower for the hydrolysis of the P(+)-isomers than for the P(−)-isomers. In addition it is shown that, for

Table 1. Hydrolysis rate constants (min^{-1}, ±95% confidence limits) of C(+)P(±)- and C(−)P(±)-soman (50 nM) in various media, at 37° and pH 7.4–7.6

Fraction	Soman isomer C(+)P(−)	C(+)P(+)	C(−)P(+)	C(−)P(−)
Plasma/Tris*	0.016 ± 0.005	3.8†	0.446 ± 0.006	0.027 ± 0.006
Tris buffer	0.003 ± 0.001	0.004 ± 0.003	0.003 ± 0.002	0.004 ± 0.002
Albumin/Serum ultrafiltrate‡	0.010 ± 0.002	0.014 ± 0.002	0.010 ± 0.001	0.014 ± 0.001
Fraction IV-1§/Tris	0.003 ± 0.002	0.006 ± 0.002	0.003 ± 0.002	0.004 ± 0.002
Fraction IV-1§/Tris‖	0.007 ± 0.001	0.012 ± 0.002	0.005 ± 0.001	0.005 ± 0.001

* 50% (v/v).
† Obtained by extrapolation (Arrhenius plot) of values calculated at lower temperatures.
‡ 4% (w/v) serum albumin/serum ultrafiltrate (30.000 NMWL) is further diluted with Tris to 50% (v/v).
§ 0.5% (w/v).
‖ 2.3 mM Ca^{2+} added.

Table 2. Activation energies* for the hydrolysis of soman† stereoisomers

Fraction	Soman isomer C(+)P(−)	C(+)P(+)	C(−)P(+)	C(−)P(−)
Plasma/Tris	52 ± 12	34 ± 3	35 ± 3	58 ± 9
Tris	55 ± 13	57 ± 8	55 ± 13	57 ± 8
Fraction IV-1/Tris‡	—	54 ± 10	—	—

* kJ mol^{-1}; ±95% confidence limits; obtained from the Arrhenius-plot (Figure 2; not shown for fraction IV-1/Tris).

† C(+)P(±)-, C(−)P(±)-soman is used with Plasma/Tris; C(±)P(±)-soman is used with Tris.

‡ 2.3 mM Ca^{2+} added.

the P(−)-isomers, there is no difference between activation energies of hydrolysis in Tris and in plasma/Tris. Thus, in plasma the P(+)-isomers are selectively hydrolysed according to an enzymatic mechanism with a correspondingly lower activation energy. Experiments were done in undiluted plasma with C(+)- and C(−)-soman at pH 7.2 to 7.5 and at various temperatures. Similar values for the activation energies were found, i.e. for C(−)P(+)-soman 32.2 ± 3.3 kJ/mol and for C(+)P(+)-soman 32.2 ± 5.0 kJ/mol. In undiluted human serum and at a pH 8.7 to 8.8 the activation energy of C(−)P(+)-soman was calculated from k values obtained with the indirect method, using C(±)P(±)-soman [1]. A value of 29.7 ± 9.2 kJ/mol was found. These findings indicate that a twofold dilution with Tris does not basically influence the enzymatic mechanism (E_a). The lower activation energy is not found for the P(−)-isomers, suggesting a hydrolysis mechanism different from the enzymatic hydrolysis of the P(+)-isomers.

Inhibition of catalytic activity. The role of Ca^{2+}

The inhibition of hydrolytic activity was investigated primarily using preincubated human serum and C(±)P(±)-soman, quantified as the summation of four isomers. Iodoacetate, a known inhibitor of SH-groups did not affect the activity to a significant extent as can be seen from Table 3, but the addition of chelators of divalent ions such as 8-hydroxy-quinoline and ethylenediamine tetraacetic acid (EDTA) results in an important inhibition. Aurin tricarboxylic acid ammonium salt (aluminon) did not produce any effect on its own (Table 3) but, in combination with EDTA, an almost complete inhibition of degradation was obtained (Table 4). To see whether the inhibition could be attributed to a chelation of Ca^{2+}, the hydrolysis of C(+)P(+)- and C(−)P(+)-soman was followed in preincubated serum to which EDTA (10 mM) was added, resulting in an important inhibition during the first minute(s). The activity, however, returned when Ca^{2+} (10 mM) (Fig. 3) was added, suggesting that Ca^{2+} may act as cofactor of the soman-degrading enzyme(s).

Influence of other divalent metal ions

From Table 5 it is clear that neither Ba^{2+}, Co^{2+}, Sr^{2+} nor Mn^{2+} affect serum phosphorylphosphatase activity towards C(+)P(+)- and C(−)P(+)-soman. Hg^{2+} strongly inhibits the enzymatic hydrolysis of both P(+)-isomers but the hydrolysis of the P(−)-isomers is not significantly influenced by Hg^{+}, suggesting a different hydrolysis mechanism for the P(+)- and P(−)isomers respectively.

pH dependency

Figure 4 shows the influence of pH on the hydrolysis rate constants of C(+)P(+)- and C(−)P(+)-soman in serum. It is seen that, in the pH-interval 6.5–10.5, the rate constant of C(+)P(+)-soman does not change significantly, whereas the rate constant of C(−)P(+)-soman increases, in a sigmoid way,

Table 3. Influence* of some inhibitors† on the degradation of C(±)P(±)-soman‡ in preincubated§ human serum

Inhibitor concentration (mM)	% Remaining soman after 10 min‖ Control	Iodoacetate	8-OH-quinoline	EDTA	Aluminon
—	35	—	—	—	—
1	—	33	37	39	32
10	—	36	52	78	36
100	—	47	93	88	37

* % Remaining soman after 10 min is compared with the control value (no inhibitor added) of 35 ± 8 (95% confidence interval).

† Inhibitor added 60 min before start of degradation.

‡ 50 nM initial concentration; C(±)P(±)-soman (=sum of the two peaks) is determined; pH 8.7–8.8; 25°.

§ Preincubated with 2.5 μM C(±)P(±)-soman at 25° during 17 hr.

‖ Each value is the mean of at least two determinations.

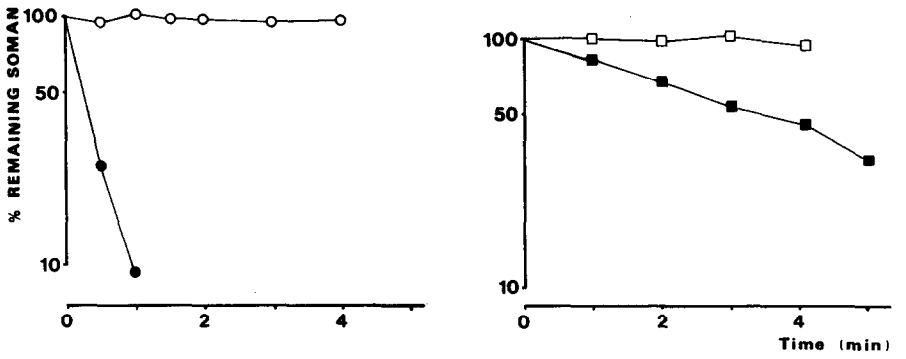

Fig. 3. Hydrolysis of soman in human serum at pH 7.4 and 25°. Open symbols human serum + EDTA (10 mM). Filled symbols human serum + EDTA (10 mM) + Ca^{2+} (10 mM). ●, ○ C(+)P(+); ■, □ C(−)P(+).

with increasing pH. In Fig. 5 the pH stability of the enzyme(s) involved is shown: the hydrolysis rate constants are determined at pH 8 but, prior to the hydrolysis, the serum was kept at the indicated pH for 60 min. It is clear that somanase is stable between pH 6 and 10.5. The resulting bell-shaped curves are similar for both substrates.

DISCUSSION

In this paper an attempt is made to evaluate the contributions of various serum fractions to the total hydrolysis rate constants of the four isomers of soman in human serum and plasma.

Since first-order conditions apply [1], the rate constants obtained in various fractions can be added, provided a correction for the spontaneous hydrolysis in Tris. From Table 1 it is clear that, for the P(+)-isomers, the contributions of the serum fractions are negligible as compared with the hydrolysis in plasma/Tris, since they account for about half of the total hydrolysis of the C(−)P(−)-isomer and nearly completely for C(+)P(−)-soman. In previous experiments with human serum and serum ultrafiltrate [1] it was shown that a protein-dependent mechanism is responsible for the enantio-selective hydrolysis of the P(+)-isomers of soman. Table 2 shows that, in comparison with the spontaneous hydrolysis of soman in Tris, the hydrolysis of both C(−)P(+)- and C(+)P(+)-soman is characterized by a lower activation energy, which is in agreement with an enzymatic mechanism. Since a corresponding lowering in activation energy is not found for the P(−)-isomers it can be concluded that such an enzymatic reaction does not apply to the hydrolysis of these isomers. Indeed, for the C(−)P(−)isomer, where the contributions of the fraction IV-1 and albumin do not fully account for the total hydrolysis (Table 1), the effect of an important lowering in activation energy by some 16 kJ/mol should be reflected in the E_a of the total hydrolysis, and such an effect is not seen on Table 2. Together, these findings point

Table 4. Influence* of the combination of EDTA/Aluminon† on the hydrolysis of C(±)P(±)-soman‡ in preincubated§ human serum

	% Remaining C(±)P(±)-soman after 10 min‖			
		EDTA (mM)		
Aluminon (mM)	—	0.1	1	10
—	35	—	—	—
1	—	36	37	74
10	—	36	40	87
100	—	not determined	43	95

* % Remaining C(±)P(±)-soman (50 nM initial concentration) after 10 min is compared with the control value (no inhibitor added) of 35 ± 8, 95% confidence limit).

† Added 60 min before the start of the hydrolysis experiment.

‡ Total soman (=sum of the two peaks) is determined; pH 8.7–8.8; 25°.

§ Preincubated with 2.5 μM C(±)P(±)-soman; 17 hr; 25°.

‖ Each value is the mean of at least two determinations.

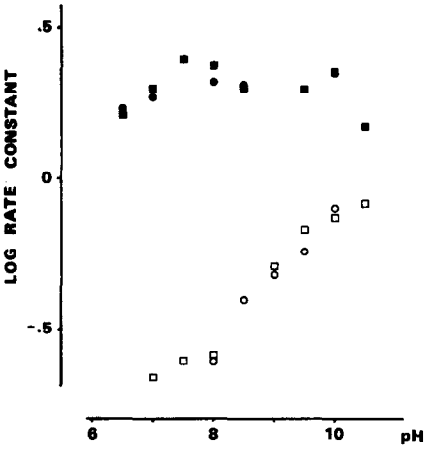

Fig. 4. pH dependence of the hydrolysis rate constants of the C(±)P(+)-isomers of soman in human serum at 25°. Filled symbols C(+)P(+). Open symbols C(−)P(+). For each isomer two serum batches were used (○, □ and ●, ■).

Table 5. Influence* of divalent metal ions (1 mM) on the hydrolysis of C(+)P(±)- and C(−)P(±)-soman (2.8 μM) in preincubated† human serum (pH 7.4, 25°)

Metal ion (1 mM)	k/k_{ref}			
	C(−)P(+)	C(−)P(−)	C(+)P(+)	C(+)P(−)
Co^{2+}	0.91	—	0.94	—
Ba^{2+}	0.99	—	0.96	—
Sr^{2+}	0.87	—	0.99	—
Mn^{2+}	0.83	—	0.80	—
Hg^{2+}	0.33	1.09	0.20	0.82

* Expressed as k/k_{ref}, with k_{ref} the first-order rate constant, without ions added, and k, the first-order rate constant with ions added.

† Preincubated with 2 μM of C(±)P(±)-soman at 25°, 17 hr.

towards important mechanistic differences between the hydrolysis of the P(+)- and the P(−)-isomers in human plasma. An explanation of the rate enhancement in this case and in the case of the remaining activity of C(−)P(−)-soman in Plasma/Tris may be found in a binding of soman to proteins. Such binding should lead to a rate enhancement on the basis of changes in entropy between a unimolecular (bound) and the (unbound) spontaneous bimolecular reaction, the so-called proximity effect [24].

In serum fraction IV-1, C(+)P(+)-soman is hydrolyzed more rapidly than the other isomers (Table 1). This could be due to somanase precipitated with the serum fraction. But proposing that the same enzyme is hydrolyzing both P(+)-isomers, a proportional enhancement would be expected for the C(−)P(+)-isomer. Considering the measurement error, such an enhancement could not be assessed. The temperature dependency of the hydrolytic activity of fraction IV-1 towards C(+)P(+)-soman (not shown) allows the calculation of an activation energy of 54.0 ± 10.9 kJ/mol (Table 2), which does not significantly differ from the activation energies found for non-enzymatic hydrolysis of soman.

With regard to the hydrolysis of soman isomers in Tris it can be noticed that Larsson [25] found an E_a-value of 38.1 kJ/mol for the hydrolysis of sarin at pH 9. This value is much lower than the corresponding values for the spontaneous hydrolysis of the soman isomers. An explanation for this difference may be found by considering steric factors of the bipyramidal transition state complex: steric hindrance is supposed to be more important for the pinacolyl- than for the isopropyl-moiety.

Some A-esterases, e.g. DFP-ase [26] and tabunase [27] are inhibited by compounds which react with sulphydryl groups, suggesting that these groups are needed to hold the enzyme in an active conformation. Iodoacetate, a known SH-inhibitor, hardly inhibits the somanase activity, even at 0.1 M (Table 3). On the contrary the addition of Hg^{2+}-ions at 1 mM inhibits significantly the hydrolytic effect (Table 5). Probably iodoacetate is too large to react with SH-groups necessary for somanase activity. Apparently, the use of 8-hydroxyquinoline and ethylenediamine tetracetic acid (EDTA) as inhibitors, was more successful (Table 5). From these results it can be concluded that divalent ions are necessary co-factors to the enzymatic process.

The hydrolysis of phosphonate esters in human plasma is inhibited by EDTA [10]. Figure 3 shows that the hydrolysis of both C(+)P(+)- and

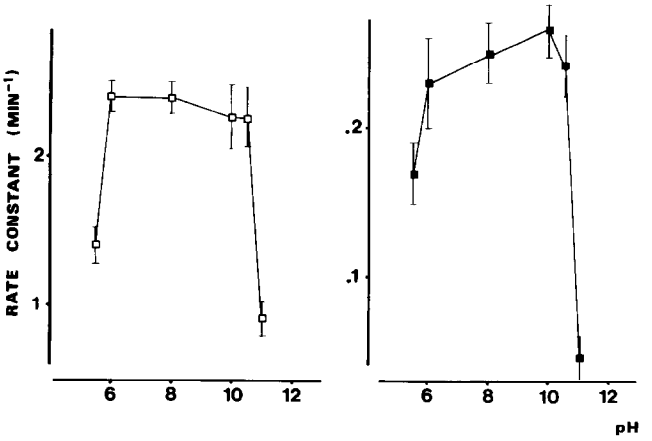

Fig. 5. pH dependency of the hydrolysis rate constants of the C(±)P(+)-isomers of soman in human serum. The serum is kept at the pH indicated and at 25° for 30 min and subsequently the hydrolysis rate constants are determined at pH 8 and 25°. □, C(+)P(+), ■, C(−)P(+). 95% confidence limits are indicated.

C(−)P(+)-soman is inhibited by the addition of 10 mM EDTA in preincubated human serum. Addition of 10 mM Ca^{2+}-ions recovered the activity, suggesting that calcium ions play a role as a co-factor of somanase.

According to [5], barium and strontium ions activate serum phosphorylphosphatase activity, whereas cobalt and manganese ions inhibit this enzyme activity. Table 5 shows that, for somanase, the activity is not significantly influenced by these ions. On the other hand Hg^{2+}-ions strongly inhibit the enzymatic hydrolysis of both P(+)-isomers, in accordance with previous observations on the hydrolysis of sarin in plasma [9]. The hydrolysis of the P(−)-isomers, however, is not significantly affected by Hg^{2+}. These findings are indications for fundamental differences in somanase-catalysed hydrolysis, between P(+)- and P(−)-isomers, in human plasma and serum.

An evaluation of the pH-dependency of enzymatic activity might give some indication whether only one or several enzymes are involved in the enzymatic degradation of soman in serum. Figure 4 shows that, in the pH-interval studied, the hydrolysis of C(+)P(+)-soman remains practically unaffected, whereas the hydrolysis of the C(−)P(+)-isomer is enhanced with raising pH. Since the P(+)-isomers are also diastereoisomers, one can expect that an enzyme conformation, if modified with raising pH, will favour the hydrolysis of only one diastereoisomer. Thus from these results it cannot be concluded that more than one enzyme exists with somanase activity. Somanase-activity appears to be stable for both P(+)-substrates between pH 6 and 10.5 (Fig. 5). Neither from this part of the study it can be concluded that more than one enzyme is involved in the hydrolysis of soman in human serum.

Acknowledgement—We thank Dr H. P. Benschop and Dr L. P. A. De Jong, Prins Maurits Laboratory—TNO, for helpful discussions.

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