

Direct reaction of oximes with crotylsarin, cyclosarin, or VX in vitro

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Abstract The direct reaction of seven pyridinium oximes with the organophosphorus compounds (OPCs) crotylsarin, cyclosarin, and VX was studied by spectrophotometry. This method allows to quantify different parameters: (a) the half-life times ($t_{1/2}$) of the oxime-OPC reactions on the basis of the changes in the absorption at the zwitterion (betaine) peak maximum, (b) the first- and second-order rate constants (k_1 , k_2), and (c) the maximum reaction velocities (v_{\max}). The results of the study show that the reaction velocity of the nerve agents with any of the oximes investigated decreased in the order crotylsarin > cyclosarin > VX. The comparison of the reaction rates of the three therapeutically used oximes (2-PAM, obidoxime, HI 6) with the respective OPC gave the highest rate for crotylsarin and cyclosarin with obidoxime and to a similar degree with HI 6, while in the case of VX the most reactive oxime was HI 6. The reaction velocity of the nerve agents with the monopyrindinium oxime 2-PAM was lower as compared to the bispyridinium oximes (obidoxime, HI 6). The results obtained with the two sarin analogues indicate that the direct reaction with 2-PAM, obidoxime, or HI 6 could be used for non-corrosive decontamination purposes, especially, if sensitive biological surfaces like skin, mucous membranes, or wounds are considered. However, in view of the concentrations of nerve agents and oximes, which could be expected during OPC poisoning in man, the maximum reaction velocities would not be high enough to contribute markedly to the detoxication of nerve agents in vivo.

Keywords Oximes · Crotylsarin · Cyclosarin · VX · Direct reaction

Introduction

The fatal effect of poisoning by organophosphorus compounds (OPCs) like pesticides or chemical warfare agents is due to inhibition of the active site of the enzyme acetyl-cholinesterase (EC 3.1.1.7). Today it is generally accepted (Volans 1996; Lee 2003) that the treatment of OPC-poisoning is based on the immediate and simultaneous administration of an antimuscarinic (e.g. atropine), a reactivator (pyridinium oximes like 2-PAM or obidoxime), and an anticonvulsant (e.g. diazepam). In a biological environment (aqueous solution, pH 7.4) pyridinium oximes form a zwitterion (betaine) with a high electron density at the O-atom of the oximate group. This enables the oximate to react as a nucleophilic agent and to displace the phosphyl (phosphonyl or phosphoryl) residue from the enzyme. Thereby a phosphyloxime (POX) intermediate is generated. Furthermore, oximes are able to react spontaneously with a free OPC also forming a POX. This more or less stable intermediate is decomposed spontaneously into less toxic reaction products.

After the first description of the direct reaction of different PAM isomers with sarin by Green and Saville 1956, various attempts have been made to characterize and to quantify the reaction. However, the studies are hardly to compare, since the authors did not only use different methods, OPCs, and oximes, but also different buffers, pH values, temperatures, and reactant concentrations (Hackley et al. 1959; Lamb et al. 1965; Okonek 1972; Nenner 1974; Harvey et al. 1986; Waser et al.

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1992). Therefore, experiments were carried out to study the interaction of OPCs and oximes under identical conditions. In a previous communication in this journal we reported upon the direct reaction of seven pyridinium oximes (2-PAM, obidoxime, HS 6, HGA 1, HGG 12, HI 6, and HLö 7) with the OPCs sarin, soman, and tabun (Becker et al. 1997). This work was continued and is presented here giving the results of the interaction studies of the same seven pyridinium oximes with the OPCs cyclosarin, crotylsarin, and VX. Taken together, experiments with 42 different combinations of oximes and OPCs were carried out and described. Since these studies were performed under identical conditions (spectrophotometry, 0.1 M MOPS buffer, pH 7.4 at 25°C, oxime 15 µM, nerve agent 1.5 mM), the results are comparable.

The aim of the work was to elucidate whether the direct reaction could contribute to the therapeutic effect of oxime treatment after OPC-poisoning. Furthermore, there was the question whether the oximes could serve as non-corrosive decontaminating compounds, especially for sensitive biological surfaces like skin, mucous membranes, or wounds.

Materials and methods

Chemicals

Crotylmethylphosphonofluoridate (crotylsarin, 97% pure), cyclohexylmethylphosphonofluoridate (cyclosarin, > 98% pure), and O-Ethyl S-2-(diisopropylamino)ethyl methylphosphonothiolate (VX, 98% pure) were made available by the German Ministry of Defence (purity checked by gas chromatography). Morpholinopropanesulfonic acid (MOPS) and pyridinium-2-aldoxime methiodide (2-PAM iodide or pralidoxime iodide) were obtained from Sigma (Deisenhofen, Germany).

HGG 12 (pyridinium, 1-[[3-(benzoyl-pyridinio)methoxy]methyl]-2-[(hydroxyimino)methyl]dichloride or dinitrate) and obidoxime (1,1'-[oxy-bis(methylene)]bis[4-(hydroxyimino)methyl]pyridinium dichloride) were obtained from Merck (Darmstadt, Germany). HI 6 (pyridinium, 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]dichloride) was a gift of Dr. J. G. Clement (DRES, Ralston, Alberta, Canada). HLö 7 (pyridinium, 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2,4-bis[(hydroxyimino)methyl]dimethanesulfonate) was obtained from J. Braxmeier (Chemisches Laboratorium, Döpshofen, Germany). HGA 1 (3-(N,N-dimethylamino-carbonyloxy)-2-(hydroxyiminomethyl)-1-methylpyridinium iodide) was

synthesized by Dr. H. Eckert (Institut für Organische Chemie I, TU München, Germany), and HS 6 (pyridinium, 1-[[[3-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]dichloride) was obtained from Dr. H. Oldiges (Fraunhofer-Institut für Umweltchemie und Ökotoxikologie, Schmallingenberg, Germany).

Solutions

Stock solutions of the oximes (30 µM) and OPCs (3 mM crotylsarin, cyclosarin, or VX) were freshly prepared in 0.1 M MOPS buffer, pH 7.4 at 25°C immediately before use. To facilitate the dispersion and solution of VX in the buffer, the preparation was shaken for 2 min by means of a Vortex mixer (Vortex Genie 2, step 6).

UV spectra

The oxime spectra (250–430 nm) were recorded using a 1-cm quartz cuvette and a UV-VIS Beckman type DU 70 instrument with temperature control. The reference spectrum of 15 µM oxime (in the absence of OPC) was determined in 0.1 M MOPS buffer, pH 7.4 at 25°C. Immediately after mixing of 1.5 ml of oxime solution with 1.5 ml of the OPC stock solution in the quartz cuvette, the reaction spectra, i.e. the time dependent changes of the oxime concentrations were followed at 25°C after various reaction time intervals (0.25–540 min). At the start of the reaction the final concentrations of each of the oximes and OPCs were 15 µM and 1.5 mM, respectively, i.e. the oxime to OPC ratio was 1:100. In the spectral region of interest neither MOPS buffer nor the OPCs showed any absorbance. The peak absorbance in the region of interest (250 nm–400 nm), representing the zwitterionic (betaine) form of the oxime (Schoene 1973; Waser et al. 1992), was used for quantification and calculation of the decomposition half-life times $t_{1/2}$, the first-order rate constants k_1 , the second-order rate constants k_2 , and the maximum reaction velocities v_{\max} at the reactant concentrations indicated.

Calculations

The semilogarithmic plot of oxime concentrations versus time showed a straight line indicating a monomolecular reaction (pseudo-first-order kinetics). After the graphic determination of the half-life time $t_{1/2}$ the first-order rate constant k_1 is available, since at that point of time the initial oxime concentration is reduced to $1/2$:

$$k_1 \times t_{1/2} = -\ln 1/2 = -2.303 \log 1/2$$

$$t_{1/2} = -1/k_1 \times 2.303 \times (-0.301) = 0.693/k_1 \text{ (min)}$$

for practical use: $k_1 = 0.693/t_{1/2} \text{ (min}^{-1}\text{)}$

k_1 leads to the second-order rate constant k_2 :

$$k_2 = k_1/(\text{initial OPC concentration}) = k_1/1.5 \times 10^{-3} \text{ (M}^{-1}\text{ min}^{-1}\text{)}$$

By means of k_2 the maximum reaction velocity can be calculated:

$$v_{\max} = k_2 \times (\text{oxime concentration}) \times (\text{OPC concentration}) \text{ (M min}^{-1}\text{)}$$

$$v_{\max} = k_2 \times (15 \times 10^{-6}) \times (1.5 \times 10^{-3}) \text{ (M min}^{-1}\text{)}$$

for practical use: $v_{\max} = k_2 \times 22.5 \times 10^{-9} \text{ (M min}^{-1}\text{)}$

All kinetic parameters summarized in Table 1 were calculated this way.

Results

The direct reaction of the oxime HI 6 and crotylsarin is shown in Fig. 1. The spectrum of HI 6 (reference in the

absence of crotylsarin) is characterized by three maxima at 273, 300, and 354 nm, respectively. After addition of an 100-fold excess of crotylsarin, a marked but transient increase of the peak at 273 nm over a period of 10 min could be observed, whereas the two other maxima decreased. The half-time of HI 6 concentration decrease was 3.7 min according the diminution of the zwitterion peak at 354 nm.

Figure 2 shows the direct reaction of HI 6 and VX. It is obvious that the change of the HI 6 spectrum was different, compared to the experiments with crotylsarin. The maxima at 300 and 354 nm decreased similar to the experiments with crotylsarin, whereas the peak at 273 nm was transformed into a shoulder. On the basis of the decreasing absorbances at 354 nm the half-life time of HI 6 decrease was determined to be 16.4 min.

The spectrum of 2-PAM (reference in the absence of cyclosarin) displays a peak at 294 nm and a shoulder (zwitterion) at 330 nm (Fig. 3). After addition of an 100-fold excess of cyclosarin the maximum and the shoulder decreased with increasing reaction time. The

Table 1 Decomposition half-lives ($t_{1/2}$), rate constants (k_1 , k_2), and maximum reaction velocities (v_{\max}) of different oximes (15 μM) in the presence of 1.5 mM of crotylsarin, cyclosarin, or VX in 0.1 M MOPS buffer, pH 7.4 at 25°C

Oxime	Poison	$t_{1/2}$ (min)	k_1 (min ⁻¹)	k_2 (M ⁻¹ min ⁻¹)	v_{\max} (M min ⁻¹)
2-PAM iodide ($\lambda = 294$ nm)	Crotylsarin	5.3	0.1307	87.2	1.961×10^{-6}
	Cyclosarin	7.5	0.0924	61.6	1.386×10^{-6}
	VX	66	0.0105	7.0	0.158×10^{-6}
2-PAM iodide ($\lambda = 326$ nm)	Crotylsarin	3.9	0.1777	118.4	2.664×10^{-6}
	Cyclosarin	6.0	0.1155	77.0	1.733×10^{-6}
	VX	76	0.0091	6.1	0.137×10^{-6}
Obidoxime ($\lambda = 354$ nm)	Crotylsarin	3.5	0.1980	132.0	2.970×10^{-6}
	Cyclosarin	5.8	0.1195	79.7	1.793×10^{-6}
	VX	42.5	0.0163	10.9	0.245×10^{-6}
HS 6 ($\lambda = 354$ nm)	Crotylsarin	4.2	0.1650	110.0	2.475×10^{-6}
	Cyclosarin	8.0	0.0866	57.7	1.298×10^{-6}
	VX	20.2	0.0343	22.9	0.515×10^{-6}
HGA 1 ($\lambda = 340$ nm)	Crotylsarin	5.9	0.1174	78.3	1.761×10^{-6}
	Cyclosarin	10.9	0.0636	42.4	0.954×10^{-6}
	VX	>540	—	—	—
HGG 12 chloride ($\lambda = 356$ nm)	Crotylsarin	4.8	0.1443	96.2	2.165×10^{-6}
	Cyclosarin	7.6	0.0912	60.8	1.368×10^{-6}
	VX	24.6	0.0282	18.8	0.423×10^{-6}
HGG 12 nitrate ($\lambda = 356$ nm)	Crotylsarin	4.4	0.1575	105.0	2.362×10^{-6}
	Cyclosarin	6.8	0.1019	67.9	1.528×10^{-6}
	VX	26.8	0.0258	17.2	0.387×10^{-6}
HI 6 ($\lambda = 354$ nm)	Crotylsarin	3.7	0.1873	124.9	2.809×10^{-6}
	Cyclosarin	5.9	0.1175	78.3	1.762×10^{-6}
	VX	16.4	0.0422	28.2	0.630×10^{-6}
HLö 7 ($\lambda = 354$ nm)	Crotylsarin	6.7	0.1034	68.9	1.551×10^{-6}
	Cyclosarin	10.5	0.0660	44.0	0.990×10^{-6}
	VX	>540	—	—	—
HLö 7 ($\lambda = 379$ nm)	Crotylsarin	9.1	0.0761	50.8	1.142×10^{-6}
	Cyclosarin	12.2	0.0568	37.9	0.853×10^{-6}
	VX	151	0.0046	3.1	0.070×10^{-6}

Calculations are on the basis of the absorbances at the wavelengths indicated in parentheses. The full chemical name of the oximes is given in [Materials and methods](#)

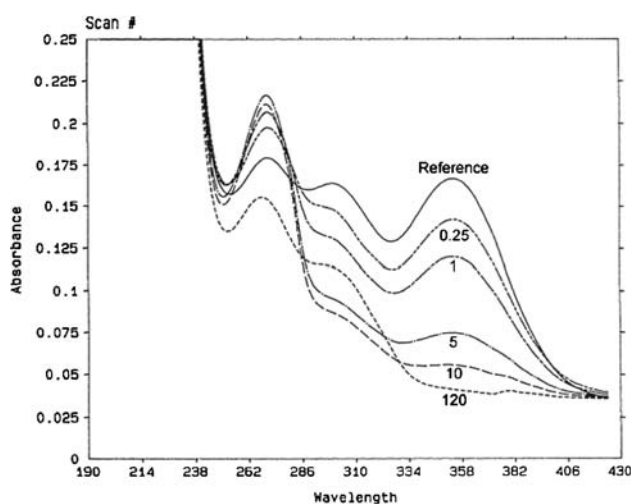


Fig. 1 Time related spectral changes of a mixture of HI 6 (15 μM) and crotylsarin (1.5 mM) in 0.1 M MOPS buffer, pH 7.4 at 25°C. Time of recording relative to the reaction start by mixing of the components (HI 6, 30 μM + crotylsarin, 3 mM, in 0.1 M MOPS buffer, 1.5 ml each) given in minutes. For the full chemical name of the oxime see [Materials and methods](#)

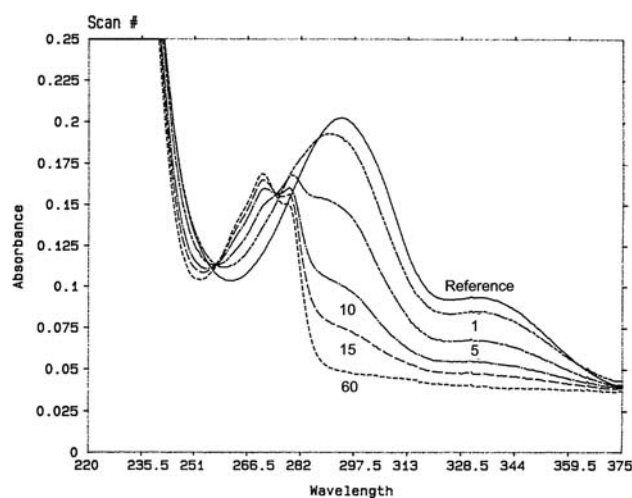


Fig. 3 Time related spectral changes of a mixture of 2-PAM (15 μM) and cyclosarin (1.5 mM) in 0.1 M MOPS buffer, pH 7.4 at 25°C. Time of recording relative to the reaction start by mixing of the components (2-PAM, 30 μM + cyclosarin, 3 mM, in 0.1 M MOPS buffer, 1.5 ml each) given in minutes. For the full chemical name of the oxime see [Materials and methods](#)

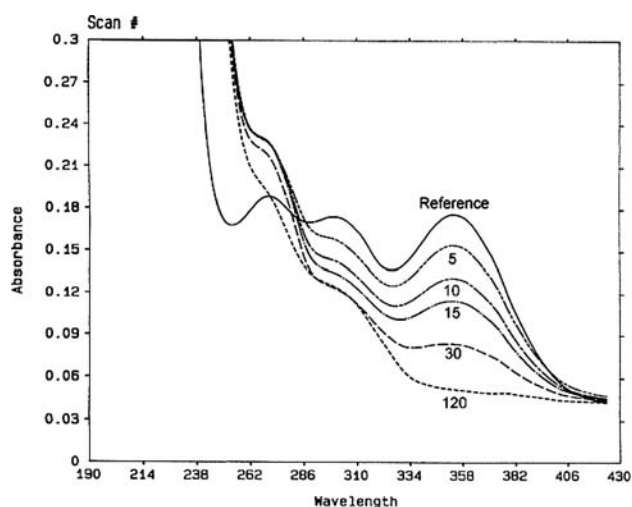


Fig. 2 Time related spectral changes of a mixture of HI 6 (15 μM) and VX (1.5 mM) in 0.1 M MOPS buffer, pH 7.4 at 25°C. Time of recording relative to the reaction start by mixing of the components (HI 6, 30 μM + VX, 3 mM, in 0.1 M MOPS buffer, 1.5 ml each) given in minutes. For the full chemical name of the oxime see [Materials and methods](#)

peak at 294 nm showed a hypsochromic shift during the incubation time, while the shoulder was stationary. The half-life time of 2-PAM was 7.5 min (294 nm) or 6.0 min (326 nm), respectively.

The experimental results and the calculated kinetic parameters are summarized in Table 1. As the dimension (min^{-1}) of k_1 in Table 1 shows, this value characterizes the decay of a substance (OPC) per minute. The first-order rate constant k_1 is independent of the con-

centration. The multiplication of k_1 with 100 gives the percentage of decay per minute; for example, the first line in Table 1 shows a k_1 value of 0.1307 min^{-1} , that means a disappearance per minute of 13.07% of crotylsarin. In contrast to k_1 the second-order rate constant k_2 is dependent on the concentration. The knowledge of k_2 is necessary for the calculation of the maximum reaction velocity v_{max} . This parameter cannot be exceeded and describes the maximum quantity of a substance (moles per litre) that react per minute.

It can be taken from Table 1 that the reaction velocities increased in the order $\text{VX} < \text{cyclosarin} < \text{crotylsarin}$. The reaction of VX and HGA 1 was too slow ($t_{1/2} > 540 \text{ min}$) to calculate a half-time. Even the doubling of the VX concentration from 1.5 to 3.0 mM did not increase the reaction velocity. The same phenomenon was to be observed ($t_{1/2} > 540 \text{ min}$) with the reaction of VX and HLö 7 (354 nm; Table 1).

Discussion

The results indicate that the oximes investigated react spontaneously with the three OPCs. The reaction velocities, however, were markedly different. The sarin analogues showed high reaction velocities with half-times of the oximes between 3.5 and 9.1 min (crotylsarin) and between 5.8 and 12.2 min (cyclosarin). In contrast, the decrease of the oxime concentration was much slower in the presence of VX (half-times

between 16.4 and 151 min). In two cases, HGA 1 at 340 nm and HLö 7 at 354 nm, the direct reaction with VX was too slow to calculate half-lives of the oximes ($t_{1/2} > 540$ min). The explanation might be that the leaving group of the sarin analogues is the small fluoride ion (MW ca. 19 Da) whereas the leaving group of VX (the anion of diisopropylaminothioethanol) shows more than the eightfold mass (MW ca. 160 Da). Other reasons for the different behaviour of the sarin analogues and VX might be different water solubility, different electron density at the P-atom, and steric factors.

The direct reaction is accomplished at least in three steps, (a) generation of the oxime zwitterion (betaine), (b) formation of a phosphoxime (POX) intermediate, and (c) irreversible decomposition of the POX into less toxic compounds (Steinberg and Solomon 1966). POXs were identified as highly toxic anticholinesterases, even 10- to 100-fold more toxic than their parent compounds (Hackley et al. 1959; Lamb et al. 1964, 1965; Schoene 1973; Nenner 1974; de Jong and Ceulen 1978; Harvey et al. 1984, 1986; Worek et al. 2000). The stability of the POX intermediate seems to be an important factor determining the velocity of the direct reaction. During the reaction of HI 6 and crotylsarin, an immediate increase followed by a diminution of the peak at 273 nm was observed (Fig. 1). This could be interpreted as formation and degradation of a metastable transition state complex in which the POX and other reaction products are involved. In the same way the alteration of the HI 6 spectrum at 273 nm during the reaction with VX (Fig. 2) and the marked hypsochromic shift during the reaction of 2-PAM and cyclosarin (Fig. 3) could be explained.

Recently, Ashani et al. (2003) reported upon the distinctive properties of the POXs formed from 2-, 3-, and 4-PAM with different OPCs (e.g. sarin, cyclosarin, soman, tabun, VX). The $t_{1/2}$ values of the non-enzymatic decomposition of the 2-PAM POXs ranged from 0.7 to 1.5 min. We observed similar half-lives from 3.9 to 7.5 min for the complete direct reaction (formation and decomposition of the POXs) of cyclo- or crotylsarin with 2-PAM. However, our results obtained with 2-PAM and VX at 294 and 326 nm show much higher values ($t_{1/2} > 60$ min). The reason for this difference might be the leaving group effect during POX formation mentioned above. Furthermore, it must be taken in account that Ashani et al. (2003) worked with highly diluted HEPES buffer (0.01 M), pH 7.8 at 29°C. The concentration of the POXs was in the order of magnitude of 0.05 mM (=50 µM) as described in an earlier paper of this group (Leader et al. 1999). Compared to the conditions we used (0.1 M MOPS buffer, pH 7.4 at

25°C; 15 µM oxime and 1.5 mM nerve agent), the different buffer type and buffer concentration, pH value, and reaction temperature may have an accelerating effect on the decomposition velocity of the POXs and may explain the short half-times given by Ashani et al. (2003).

The question whether the direct reaction could contribute to the detoxication of nerve agents in vivo during OPC poisoning can be evaluated by means of the maximum reaction velocities (v_{\max}) in Table 1. These values reach from 0.070×10^{-6} to 2.970×10^{-6} M min⁻¹, hence, the pair HLö 7/VX shows the lowest and the combination obidoxime/crotylsarin the highest reaction velocity (Table 1). It can be assumed that during poisoning the OPC concentration in vivo is in the nanomolar range and the reasonable therapeutic concentration of an oxime is approximately 15 µM. Under these conditions the maximum reaction velocities are $v_{\max} = 0.465 \times 10^{-12}$ M min⁻¹ (at 10 nM VX and 15 µM HI 6) and $v_{\max} = 19.80 \times 10^{-12}$ M min⁻¹ (at 10 nM crotylsarin and 15 µM obidoxime).

The v_{\max} values of all the other OPC oxime combinations in Table 1 are to be found within this range. These results are in agreement with a previous study (Becker et al. 1997) using soman/HI 6. In this case the maximum reaction velocity was calculated to be $v_{\max} = 2.55 \times 10^{-12}$ M min⁻¹ (at 10 nM soman and 15 µM HI 6). This order of magnitude (picomoles per litre) demonstrates that the direct reaction cannot markedly contribute to the therapeutic effect of oximes in vivo.

Conclusion

The results presented here with crotyl- and cyclosarin demonstrate that the direct reaction of OPCs and pyridinium oximes could be used for mild decontamination/detoxication purposes (skin, mucous membranes, wounds). Pyridinium oximes could substitute the efficient but corrosive preparations of hypochlorites (NaOCl solution, calcium hypochlorite/magnesium oxide powder). This holds true for previously published data obtained with sarin, soman, and tabun (Becker et al. 1997). For practical administrations it might be useful to accelerate the third step of the direct reaction, i.e., the degradation of the POXs, by addition of bacterial (Ashani et al. 1998; Leader et al. 1999; Herkenhoff et al. 2004) or human plasma enzymes (Kiderlen et al. 2000; Worek et al. 2000).

The importance of the direct reaction in the detoxication during human OPC poisoning may be assessed by considering the generated data (Table 1) and by

regarding the concentrations of OPCs and oximes, which can be expected in human OPC poisoning. Hence, the maximum reaction velocity was calculated to be in the range of $10^{-12} \text{ M min}^{-1}$ (picomoles per litre). This demonstrates that in vivo the direct reaction is only of small significance. However, it can be considered as a beneficial side effect to the oxime action in vivo.

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