HYDROLYTIC REACTIONS OF METHYLFLUOROPHOSPHONATES WITH CYCLODEXTRINS

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Reactions of three methylfluorophosphonates (isopropyl, 1,2,2-trimethylpropyl, and cyclohexyl) with α-, β- and γ-cyclodextrins were investigated. The cyclodextrins were found to react with stereoisomers of the methylfluorophosphonates at different rates depending on the location of the substituents at the phosphorus atom. The dependence of the rate increase on cyclodextrin concentration showed a saturation effect. The reactivity of the inclusion complexes of the cyclodextrins with the methylfluorophosphonates depended on the size of the alkyl part of the phosphonate molecule as well as on the size of the cavity of the cyclodextrins. Dependences of the reaction rates on temperature and pH of the reaction medium were recorded. Kinetic data have been used to evaluate the cyclodextrins as the catalysts.

Cyclodextrins (CD) are cyclic oligosaccharides formed by six (α-CD), seven (β-CD), and eight (γ-CD) glucopyranose units. In aqueous media their molecules form lipophilic microspaces capable of accepting lipophilic or biphilic molecules from a polar aqueous medium[1]. Hydroxy groups of CDs are partially ionized, especially in an alkaline pH region, and act thus as a nucleophile towards molecules enclosed in CD cavities, if these are sensitive to nucleophilic attack[2]. The inclusion process and subsequent nucleophilic reactions have been regarded as similar to the function of hydrolytic enzymes[1]. In the former case (Eq. (*A*)), the process yields a CD–substrate inclusion complex (CD–S) which is then converted into a complex containing a covalently bound substrate (CD–S′), which is accompanied by the cleavage of product P_1. The complex CD–S′ is then further cleaved to form product P_2 and the regenerated CD.

$$\mathrm{CD + S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{CD{-}S} \xrightarrow{k_2} \mathrm{CD{-}S' + P_1} \xrightarrow{k_3} \mathrm{CD + P_2} \qquad (A)$$

Methylfluorophosphonates (MFP), i.e. Sarin (isopropyl methylfluorophosphonate), Soman (1,2,2-trimethylpropyl methylfluorophosphonate), and Cyclosin (cyclohexyl methylfluorophosphonate), used as substrates in the present study, react with hydrolytic enzymes, e.g. alkaline phosphatases and cholinesterases, in different way. The former react as depicted in Eq. (*A*), while with cholinesterases the reaction stops at the stage of the formation of a phosphorylated enzyme[3,4]. MFPs are therefore classified as the irreversible inhibitors of these enzymes.

The aim of the present work was to propose a reaction mechanism which would describe the course of the reaction of MFPs with CDs. The CDs were taken as the model of hydrolytic enzymes.

EXPERIMENTAL

Hydrolytic reactions of MFPs were followed with an automatic titrator RTS 822 (Radiometer, Copenhagen, Denmark) working in a pH-stat regime. The instrument recorded the consumption of an aqueous 0.01 M NaOH solution neutralizing the acids evolved by the hydrolytic reactions. The initial MFP concentration was $1 \cdot 10^{-3}$ mol l^{-1}. CDs were used in the following maximum concentrations (in mol l^{-1}): Soman–β-CD $3.7 \cdot 10^{-3}$, Soman–γ-CD $15.0 \cdot 10^{-3}$, Sarin–β-CD $7.5 \cdot 10^{-3}$, and Cyclosin–β-CD $2.2 \cdot 10^{-3}$. The ionic strength of the solutions was adjusted to 0.1 mol l^{-1} by KCl.

The pseudo-first order rate constants were calculated on PC with the use of an iteration programme worked out according to ref.[5]. Dissociation and maximum rate constants were calculated similarly, using a relation analogous to the Michaelis–Menten equation (Eq. (*1*))

$$k_{obs} = k_0 + k\,[CD]/(K + [CD])\ , \qquad (1)$$

where k_{obs} is the experimental rate constant, k_0 is the rate constant of a spontaneous hydrolysis of MFP, k is a maximum rate constant, [CD] is a molar CD concentration, and K is the dissociation constant of the inclusion complex. For all the parameters determined, their standard deviations were calculated.

RESULTS AND DISCUSSION

Experimental data relating to the dependence of the reaction rate on CD concentration are summarized in Tables I and II.

For each MFP, two rate constants and two dissociation constants were calculated on analyzing the shape of the time dependence of the titrating agent consumption (Fig. 1). The sharp break of the straight line speaks for the existence of two reaction paths by which MFPs are converted into their hydrolytic products. The slopes of both straight lines document also different rates of these reactions. This finding might be ascribed to the reactions of two MFP stereoisomers, such a conclusion being based on the literature knowledge[2]. Sarin and Cyclosin occur as a mixture of two stereoisomers differing from each other by the location of their substituents on the phosphorus as a site of nucleophilic substitution. In addition to the so called reactive stereoisomerism, Soman exhibits also optical activity on the C1 atom of 1,2,2-trimethylpropyloxy group bonded to the phosphorus.

On the other hand, the above isomerism did not affect the inclusion of Soman, as its transformation, like that of Sarin and Cyclosin, proceeded by only two reaction paths.

This shows that the only stereospecific process in the reaction of Soman is nucleophilic attack and not the inclusion.

A marked difference between reactivity of S_P and R_P MFPs isomers can be accounted for by the different structure of the reaction intermediates, i.e. the inclusion complexes, formed from both stereoisomers.

With both stereoisomers, the 1,2,2-trimethylpropyl part of Soman molecule undergoes inclusion into the cavity of β-CD. This defines the position of the other substituents at the phosphorus with respect to any glucopyranose unit of the cyclodextrin ring. The next stage of the reaction involves formation of hydrogen bonding between the hydroxyl attached to the C3 atom of glucopyranose and the phosphoryl oxygen of Soman. This is followed by the interaction of ionized hydroxyl group of the C2 glucopyranose atom with the central phosphorus atom to form a covalent P–O–C bond with simultaneous release of the fluoride ion. However, this process takes place only in the case

TABLE I
Maximum hydrolysis rate constants k (min^{-1}) and dissociation constants K (mol l^{-1}) of inclusion complexes of α-, β-, and γ-CD with R and S stereoisomers of Soman (25 °C, pH 9)

CD	k_R	$k_S \cdot 10^2$	$K_R \cdot 10^4$	$K_S \cdot 10^4$
α-	4.87 ± 0.61	3.40 ± 0.10	252.0 ± 46.0	17.30 ± 2.10
β-	3.07 ± 0.22	3.75 ± 0.14	14.4 ± 2.9	5.35 ± 0.74
γ-	0.26 ± 0.01	1.08 ± 0.05	58.6 ± 0.6	6.33 ± 1.80

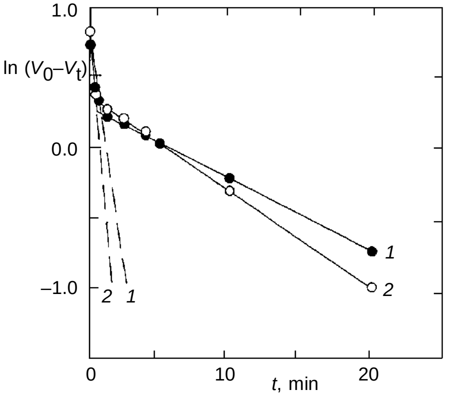

FIG. 1
Linearized time dependence of sodium hydroxide consumption in the reaction of R_P and S_P stereoisomers of *1* Soman and *2* Cyclosin with β-CD (25 °C, pH 9, [CD] $2.25 \cdot 10^{-3}$ mol l^{-1})

of R_P stereoisomers. It does not proceed with S_P stereoisomers as the fluorine atom, taking position of the methyl group of R_P stereoisomers, prevents the ionized hydroxy group from interacting with the phosphorus as the result of mutual repulsion of the negatively charged atoms. These charges thus do not allow the reaction centers to approach each other.

Calculated dissociation constants and rate constants made it possible draw some conclusions concerning the effect of the structure of MFPs and CDs on the rate of the nucleophilic reactions. From Table I, in which rate constants for the reaction of Soman with three CDs are listed, it becomes evident that the strength of the inclusion complex depends on the ratio of the size of the alkyl part of the substrate molecule to the diameter of CD cavity (Soman 0.43 nm, α-CD cavity 0.5 nm, β-CD cavity 0.8 nm, γ-CD cavity 1.0 nm). The high dissociation constants for α-CD show that the 1,2,2-trimethylpropyl group of Soman can enter the α-CD cavity only with difficulty. On the other hand, the dissociation constants for γ-CD show that the strength of the inclusion complex decreases, too, if the cavity is too great.

The values of maximum rate constants for α- and β-CDs are comparable to each other. The more significant decrease of the maximum rate constants for γ-CD documents that the reaction rate obviously decreases when the substrate is not sufficiently enclosed in the cavity.

Data on the reactivity of three MFPs with β-CD, presented in Table II, show that changes in the alkoxy part of the phosphonate molecule do not result in fundamental changes of the dissociation constant. Anomalous is only the low maximum rate constant for Sarin R_P stereoisomer. It is known[2] that the reactivity of Sarin toward α-CD is comparable with our data.

We believe that this anomaly could be explained based on analysis of the space arrangement of the inclusion complex. It is known that Sarin has significantly shorter molecule than Cyclosin and Soman (Sarin 0.68 nm, Cyclosin 0.88 nm, Soman 0.80 nm). The length of the β-CD cavity is from 0.7 to 0.8 nm. It seems likely that CD attracts the organophosphate into the cavity such that after the inclusion has been completed, the geometric centre of the cavity would overlap that of the lipophilic part of the organo-

TABLE II
Maximum hydrolysis rate constants k (min^{-1}) and dissociation constants K (mol l^{-1}) of inclusion complexes of β-CD with MFPs (25 °C, pH 9)

MFP	$k_0 \cdot 10^2$	k_R	$k_S \cdot 10^2$	$K_R \cdot 10^4$	$K_S \cdot 10^4$
Soman	1.46 ± 0.08	3.07 ± 0.22	3.75 ± 0.14	14.40 ± 2.9	5.35 ± 0.74
Sarin	2.30 ± 0.11	0.33 ± 0.02	5.92 ± 0.09	9.61 ± 4.9	4.98 ± 0.22
Cyclosin	1.81 ± 0.07	11.00 ± 0.70	5.40 ± 0.30	14.50 ± 0.2	2.52 ± 0.50

phosphate molecule. It is evident that the reaction centre (an electron deficient phosphorus) of Cyclosin and Soman lies in the plane of the hydroxy groups of the CD cavity while the centre of Sarin is immersed in the cavity below the level of the hydroxy groups. This incompability of the reaction centres could cause a decrease in the reaction rate.

The rate of nucleophilic reaction in dependence on pH of the reaction medium has been measured with β-CD–Soman inclusion complexes. The reactions of both groups of Soman stereoisomers showed increased rates with increasing pH of the reaction medium (Fig. 2). This documents that weakly acidic hydrogens of CD hydroxy groups (pK_a = 12, ref.[6]) are gradually ionized on increasing pH, yielding thus the greater amount of the reacting agent.

The last measured dependence was that of the experimental rate constant of the reaction of Soman with β-CD on the reaction temperature, measured in the 20–35 °C region. Experimental rate constants obtained have been used to calculate activation energies E_a, entropy changes ΔS, and enthalpy changes ΔH (Table III).

TABLE III
Thermodynamic parameters for the reaction of Soman with β-CD (pH 9, [CD] $2.25 \cdot 10^{-3}$ mol l^{-1})

Stereoisomer	E_a, kJ mol^{-1}	$A^a \cdot 10^{-10}$	ΔH, kJ mol^{-1}	ΔS, kJ mol^{-1}
R	78.5 ± 7.1	176.00	76.0	–0.27
S	80.2 ± 6.0	8.37	77.7	–0.63
R + *S* without CD	85.5 ± 4.5	24.70	83.3	–0.51

[a] Frequency factor of the Arrhenius equation.

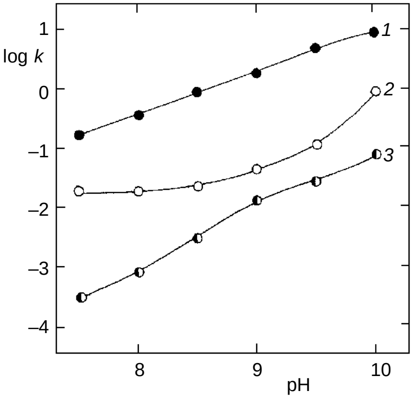

FIG. 2
pH profile of hydrolysis of Soman with β-CD. *1* R_P stereoisomer, *2* S_P stereoisomer, *3* spontaneous hydrolysis of racemate (25 °C, [CD] $2.25 \cdot 10^{-3}$ mol l^{-1})

With regard to the method used in these measurements, thermodynamic data obtained for the temperature profile of the reaction of Soman with β-CD could be applied only to the reaction as a whole and not to individual reaction steps. The enthalpy values, ΔH, show that the bond formed between the organophosphate and CD is capable of further reaction leading to phosphonic acid (compare ΔH values for the stereoisomers and spontaneous hydrolysis).

The negative values of entropy changes, ΔS, indicate that the reaction is accompanied by the restriction of the mobility of the reactant molecules. This could be caused by formation of the inclusion complex and also by that the reaction proceeds via a bimolecular mechanism.

The kinetic data obtained for the reaction of Soman with β-CD were compared with the mechanism proposed earlier for the reaction of CD with hydrolyzable substrates[1].

$$\text{CD–OH} + \text{OH}^- \rightleftharpoons \text{CD–O}^- + \text{H}_2\text{O} \qquad (B)$$

$$\text{CD–O}^- + \text{R}_1\text{OR}_2\text{POF} \longrightarrow \text{R}_1\text{OR}_2\text{POO–CD} + \text{F}^- \qquad (C)$$

$$\text{R}_1\text{OR}_2\text{POO–CD} + 2\ \text{OH}^- \longrightarrow \text{CD–O}^- + \text{R}_1\text{OR}_2\text{POO}^- + \text{H}_2\text{O} \qquad (D)$$

$$\text{R}_1\text{OR}_2\text{POF} + 2\ \text{OH}^- \longrightarrow \text{R}_1\text{OR}_2\text{POO}^- + \text{H}_2\text{O} + \text{F}^- \qquad (E)$$

Attention has been paid especially to the extent of the reaction (*D*). From the scheme it becomes clear that two equivalents of the hydroxide are consumed by the simple hydrolysis (Eq. (*E*)), one equivalent by the phosphorylation of CD for CD ionization (Eq. (*B*)), and another two equivalents of the hydroxide if the reaction involves also the regeneration step (*D*). Under the conditions used in the present work (pH 9) only one equivalent of the hydroxide seems to be consumed by the reaction, as at this pH value, CD is ionized to only very low extent (0.01%). These partial consumptions in the above reactions form then a total consumption. Under pseudo-monomolecular conditions, where CD is in a great excess and hydroxy ion concentration is maintained constant automatically, the obtained rate constant indicates that the dominant reaction by which the hydroxide is consumed in the case of *R* stereoisomers is the CD phosphorylation while in the case of *S* stereoisomers the products are formed in amounts corresponding to ratio of the rate constant of the phosphorylation to simple hydrolysis. A comparison of the final sodium hydroxide consumption for the simple hydrolysis and the CD reaction showed that the hydroxide consumption in the reaction of Soman with CD equals to ca 75 per cent of that determined for the simple hydrolysis. If Soman racemate[7] consists of 45% R_P stereoisomer and 55% S_P isomer, on using the rate constants obtained for its reaction, the hydroxide consumption would be as follows: total consumption in the simple hydrolysis is equal to 4 ml, of which 1.8 ml and 2.2 ml correponds to the S_P and R_P stereoisomer, respectively. In the reaction of Soman with CD, 1.8 ml/2 of the hydroxide corresponds to the R_P stereoisomer. In the case of the S_P stereoisomers, 2.2 ml/2 hydroxide consumption distributes between the simple hydrolysis and the reaction with CD in the ratio of their rate constants, i.e. $k_0/k_s = 1/2.5$ (i.e. 0.8 ml for the reaction with CD and 0.12 ml for the simple hydrolysis). The total volume of the titrating agent calculated from the rate constants is 2.3 ml. As the total hydroxide consumption in the reaction with CD was 3 ml, then the difference between experimental and calculated rate constant is 0.7 ml. Given that no other than (*B*)–(*E*) steps take place during the above reaction, then this volume is consumed by the reaction (*D*). However, this difference does not confirm the solely catalytic reaction course, i.e. the complete regeneration of CD. Otherwise, the hydroxide consumptions in the simple hydrolysis of Soman and of its complex with CD would be identical.

With the aim to clarify the role of catalysis in this reaction, we made experiment in which 6 equivalents of Soman were portionwise added to one equivalent of CD and we followed the consumption of the titrating agent and changes of the rate constants k_S and k_R. The results summarized in Table IV show that the hydrolytic activity of CD gradually decreases, which excludes the catalytic course of the reaction as the only process. After the addition of the sixth equivalent of Soman, the reaction most likely proceeds further as the simple hydrolysis. However, the main changes of the reaction system occur already after the third equivalent of Soman, since starting from the fourth

TABLE IV
Relative consumption of sodium hydroxide V (in %) and relative rate constants k (in %) for the reaction of one equivalent of CD with 6 equivalents of Soman (pH 9, [CD] 1 . 10^{-3} mol l^{-1})

Soman equivalent	V_t^a	V_R	k_R	k_S
1	68	100	100	100
2	67	77	40	92
3	69	33	30	78
4	79	15	0	52
5	89	4	0	44
6	100	4	0	36

[a] The total volume V_t expressed as per cent of the hydroxide consumed by the simple hydrolysis.

equivalent, the rate of the reactions of both stereoisomers cannot be distinguished. The above mentioned reaction mechanism for the R_P stereoisomers is then obviously so disturbed that in further stages it does not play any role. The question is why this does not occur before the third equivalent of Soman is added. With respect to the cooperation of the CD hydroxy groups in the hydrolysis of this isomer, the nucleophilic activity of β-CD should be exhausted only after the addition of the seventh equivalent of Soman. However, the inclusion activity of β-CD should be eliminated already after the first equivalent of Soman since the lipophilic part of one Soman molecule fills nearly completely the cavity of β-CD, as found by the analysis of a space-filling model of the inclusion complex.

The results show that the reactions of CDs with MFPs are not simple nucleophilic substitutions. The ability to differentiate the stereoisomers as well as the saturation character of the reaction course speaks for a similarity between the reactivity of CD and that of enzymes. Whether CDs exhibit also the activity of the enzymes capable of MFPs detoxification cannot be proved unambiguously based on the so far obtained results.

Further experimental work is needed to characterize the structure of the reaction system after completion of the reaction, i.e. the type of bonding between Soman and β-CD and the effect of the formed hydrolytic products on the course of the reaction of Soman in excess.

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