NMR SPECTROSCOPY OF INTERACTION OF SOME ORGANOPHOSPHORUS INSECTICIDES WITH LIPOSOMES

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The interaction of the organophosphorus insecticides GA-41, chlorophos [trichlorfon] and OKh-30 with liposomes from lecithin and a mixture of lecithin and cardiolipin has been studied by NMR spectroscopy on ^{1}H and ^{31}P nuclei. Absence of a specific interaction of chlorophos and OKh-30 with liposomes has been shown. On interacting with model membranes, GA-41 intercalates into the region of the hydrocarbon chains of the phospholipids and at particular concentrations induces the formation of certain defects in the structure of the bilayer, which leads to the penetrability of the liposomes for Pr^{3+} ions.

Organophosphorus inhibitors (OPIs), including insecticides, are substances with a neurotoxic action. In the majority of cases, OPIs inhibit the functional activity of cholinesterases [1, 2], thereby disturbing the processes of acetylcholinesterase mediation in the transfer of a nerve impulse in the central and vegetative divisions of the nervous system.

On entering the organism, the molecules of OPIs are transported to those structures to which their action is directed. The efficacy of transport and the capacity of the OPIs for selectively accumulating in certain parts of the organism must be determined by the degree of hydrophobicity and hydrophilicity of insecticide molecules [2]. Apparently, no small role in the presence of the transport and accumulation of OPIs is played the phospholipid components of biological membranes [3]. In this connection, it appeared of great interest to study the interaction of OPIs with the lipid part of the membrane using, as a model, liposomes formed from various phospholipids.

We have studied the interaction of the OPIs chlorophos [trichlorfon], GA-41, and OKh-30 with lecithin liposomes and multilamellar dispersions of a mixture of lecithin and cardiolipids by NMR spectroscopy on ^{1}H and ^{31}P nuclei. At physiological pH values, cardiolipin has a negative charge. The use of samples made from a mixture of two lipids permitted the influence of negatively charged lipids on the process involved in the interaction of OPIs with bilayers to be revealed. The OPIs investigated (GA-41, chlorophos, and OKh-30) differ in the degree of expression of their hydrophilic and hydrophobic properties. Chlorophos is readily soluble in water and OKh-30 moderately, while GA-41 is readily soluble in organic solvents and sparingly soluble in water. These differences must, to all appearances, determine the features of the distribution of the OPIs studied at an interphase membrane–water boundary.

Analysis of the PMR spectra of chlorophos, OKh-30, and GA-41 in buffer and with the addition of them to suspensions of lecithin liposomes showed that the presence of the liposomes caused no appreciable changes in the values of the chemical shifts and the widths of the signals of the chlorophos, GA-41, and OKh-30 molecules in the range of ratios of concentration of OPI to lipid investigated of from 0 to 3:1 (molar ratios). In their turn, the OPI molecules had practically no influence on the position and shape of the lines of the signals of the phospholipids in the liposomes.

A comparison of the times of spin-lattice relaxation T_1 of the protons of some of the functional groups of the chlorophos, GA-41, and OKh-30 molecules in buffer solution in samples of various liposomes showed the presence or absence of an interaction between the OPI molecules and the lipid bilayers. Table 1 gives values of the spin-lattice relaxation times T_1 for the methyl group (CH_3-P) of GA-41 and that of OKh-30 and for the methoxy group (CH_3–

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TABLE 1

OPI	Change in T_1			OPI	Change in T_1		
	1	2	3		4	5	6
GA-41				GA-41			
P—CH_3	1,261±0,236	0,761±0,063	0,697±0,077	P—CH_3	0,587±0,020		
N—$(CH_3)_3$		0,225±0,019	0,260±0,016	N—$(CH_3)_3$	0,186±0,013	0,235±0,025	0,312±0,080
chlorophos				chlorophos			
CH_3—O—P	2,377±0,415	2,038±0,149		CH_3—O—P			
N—$(CH_3)_3$		0,210±0,010		N—$(CH_3)_3$		0,235±0,025	
OKh-30				OKh-30			
P—CH_3	1,833±0,083	2,026±0,093		P—CH_3	1,749±0,045		
O—CH_3	3,324±0,222	4,150±0,341		O—CH_3	3,215±0,200		
N—$(CH_3)_3$				N—$(CH_3)_3$	0,292±0,007	0,235±0,025	0,322±0,008

1) OPI in buffer solution; 2) OPI in lecithin liposomes; 3) sample obtained by the cosonication of lecithin and GA-41; 4) OPI in samples obtained from lecithin and cardiolipin; 5) sample of lecithin liposomes without an OPI; 6) sample formed from cardiolipin and lecithin without an OPI.

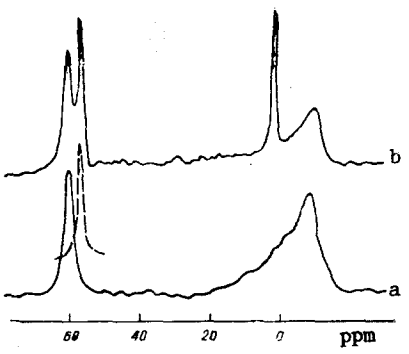

Fig. 1

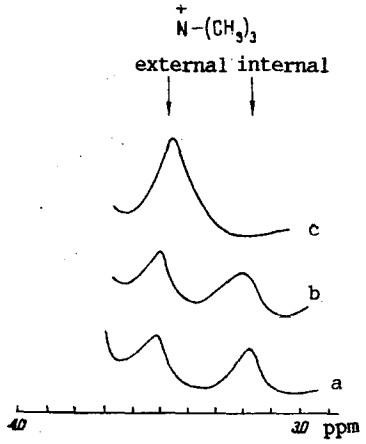

Fig. 2

Fig. 1. ^{31}P NMR spectra of aqueous dispersions of a mixture of cardiolipin and lecithin in the presence of GA-41: a) without Ca^{2+} ions; b) with a 0.5 mM concentration of Ca^{2+}; dashed line – GA-41 in buffer.

Fig. 2. ^{1}H NMR spectrum of the $N-(CH_3)_3$ fragment of the choline head of lecithin in the presence of Pr^{3+} ions without GA-41 (a) and in the presence of GA-41: b) $C_{GA-41}/C_{lipid} = 1:3$; c) $C_{GA-41}/C_{lipid} = 3:2$.

O–P) of chlorophos and that of OKh-30 and also the value of T_1 for the protons of the trimethylammonium group of the choline residue of lecithin. The value of T_1 for the methoxy group of chlorophos decreased very slightly on its addition to a suspension of liposomes. The value of T_1 for the $N-(CH_3)_3$ group of lecithin also decreased very slightly. A weak mutual influence was also detected in a mixture of liposomes with OKh-30. The results obtained indicated the absence of any specific interaction whatever of chlorophos and OKh-30 with neutral or negatively charged lipid bilayers. In the case of GA-41 in liposome samples, T_1 for the CH_3-P protons decreased in comparison with that in buffer solution by a factor of approximately 2. This effect appeared to a greater degree for liposomes from the mixture of cardiolipid and lecithin. The results obtained can be explained by the assumption that the GA-41 molecules were immersed in the lipid bilayer, which led to a restriction on their mobility. The consequence was an increase in the correlation time τ_c of the GA-41 molecules determined by the viscosity of the lipid bilayer. The absence of any appreciable influence whatever of GA-41 on T_1 of the methyl protons of the $N-(CH_3)_3$ group of the choline residue of the lecithin molecule indicated the intercalation of the $P-CH_3$ fragment of the OPI at a definite depth relative to the surface of the bilayer.

The hypothesis put forward found confirmation in the ^{31}P NMR spectra. The ^{31}P NMR spectrum of a sample containing GA-41 and liposomes formed from a mixture of cardiolipin and lecithin (at a molar ratio of 7:3) consisted of a superposition of two signals. A broad signal was characteristic for multilamellar dispersions of phospholipids [4], and an isolated signal at 58 ppm belonged to the phosphorus ion of GA-41 (Fig. 1a). A comparison of the positions and widths of this signal (without exponential weighting) with the parameters of the GA-41 signal in buffer solution showed that on interaction with liposomes the phosphorus signal of GA-41 shifted downfield by ~3 ppm and broadened substantially (by a factor of 2.5), which indicated the participation of the phosphorus atom of GA-41 in an interaction with the phospholipid molecules. We may note that it was just for these samples that the greatest change in the relaxation time of $P-CH_3$ protons of the fragment was observed.

A completely different result was obtained for the hydrophilic chlorophos molecules. The position and width of the line of the signal of the phosphorus nuclei of chlorophos in buffer solution and in the samples of liposomes had the same values. These facts confirmed the hypothesis based on PMR investigations of the absence of a specific interaction of chlorophos molecules with liposomes.

It is known [5] that Ca^{2+} ions modify the structure of bilayers formed from a mixture of cardiolipin and lecithin to a considerable degree. As investigations by the ^{31}P NMR me-

thod have shown, the modifying action of Ca^{2+} ions is also shown on disperse multilamellar structures of cardiolipin and lecithin containing GA-41. Ca^{2+} ions induce the appearance in the NMR spectra of lamellar structures of the signals of an isotropic mesophase (Fig. 1b), their intensity increasing with a rise in the concentration of Ca^{2+} ions. The signal of the phosphorus atom of GA-41 in a sample with Ca^{2+} was split into two signals (Fig. 1b), of which the narrow high-field signal corresponded to that of free GA-41 in buffer solution.

Consequently, Ca^{2+} ions, on interaction with multilamellar structures of cardiolipin and lecithin, in the first place, induce the formation of structures differing from lamellar (for example, lipid particles [6]), which agrees well with results given in the literature [7], and, in the second place, by modifying the surface of the lipid formations, displace the GA-41 molecules from the lipid phase into the aqueous phase.

In the samples with chlorophos, Ca^{2+} ions caused the appearance in the NMR spectra of the signals of an isotropic mesophase, but the signal of the phosphorus atoms of chlorophos remained unchanged. This result confirmed once more the conclusion drawn previously of the absence of an interaction of chlorophos with lipids.

It follows from the results obtained that the GA-41 molecules intercalate into the region of the acyl chains of the phospholipid bilayers. Such a localization of the molecules of OPIs in bilayers may cause a perturbation in the packing of the hydrocarbon chains of the lipids and lead to a disturbance of the integrity of the liposomes. This hypothesis was checked by the PMR method using a shift reagent, as which we chose Pr^{3+} ions. The addition of Pr^{3+} ions to samples of lecithin liposomes led to a downfield shift (Fig. 2) of the signal of the methyl protons of the $N-(CH_3)_3$ of the choline fragments of the lecithin of the external surface of the bilayer, which are accessible for Pr^{3+} ions, with no change in the positions of these signals from the internal surface of the liposomes [8, 9]. The introduction into the sample of GA-41 at a ratio of concentrations of OPI to lipid of up to 1:3 did not change the position of the signal of the $N-(CH_3)_3$ groups of the external and internal surfaces of the liposomes (Fig. 2). However, with a further increase in the concentration of GA-41 these signals approached one another, and at a ratio of the concentrations of 3:2 they fused together. It followed from this that, at certain concentrations of GA-41, Pr^{3+} ions may penetrate within the liposomes and interact with the methyl protons of the $N-(CH_3)_3$ groups present on the internal surface of the liposomes, which leads to a fusion of the signals on the "external" and "internal" $N-(CH_3)_3$ groups [8].

EXPERIMENTAL

We used egg lecithin and cardiolipin produced by the Khar'kov bacterial preparations factory. The samples of liposomes for the ^1H NMR experiments were prepared by the method described in [10]. The buffer solution (Tris-HCl, 10 mM, pH 7.5) was prepared in D_2O. The concentration of lipid in the lecithin liposomes amounted to 25 mg/ml, and in the liposomes from a mixture of lipids the weight ratio of lecithin to cardiolipin was 2:1.

Tris buffer (10 mM, pH 7.5) containing 20% of D_2O was used in the preparation of the samples for ^{31}P NMR from lecithin and cardiolipin. The multilamellar dispersions of lipids were prepared by the method described in [11]. The concentration of lipid was 70 mg/ml, and the cardiolipin/lecithin ratio 3:14.

The following OPIs were used: chlorophos [trichlorfon], GA-41 (S-n-butyl O-isopropyl methylphosphonothioate), and OKh-30 (S-n-alkylmethyl O-β-methoxyethyl ethylphosphonothioate). The GA-41 and OKh-30 had been synthesized previously by methods described in the literature [12, 13]. The chlorophos was recrystallized from benzene (100 mg from 50 ml).

NMR spectra were recorded on a Varian XL-200 spectrometer with a working frequency of 200 MHz for protons and 80.99 MHz for ^{31}P nuclei. In the recording of the ^{31}P NMR spectrum, the length of the 90° pulse was 12 μsec, the width of the spectral range 20 kHz, and the distance between pulses 0.8 sec. Broad-band "decoupling" from protons was used. To increase the sensitivity before the Fourier transformation exponential weighting with broadening of the 50-Hz line was applied. The relaxation times of the protons were determined by the method of inversion with following restoration (180°–t–90°). The length of the 90° pulse was 8.7 μsec, the time between the 180 and 90° pulse ranged from 0.05 to 5 sec, and the time of establishing equilibria was 10 sec.

CONCLUSIONS

The results obtained permit the absence of a specific interaction of chlorophos or OKh-30 either with neutral or with negatively charged liposomes to be assumed. The slight change in the value of T_1 for the $N-(CH_3)_3$ groups on the addition of chlorophos to samples of liposomes may indicate weak perturbations at the surface of the liposomes induced by the chlorophos. On interacting with model membranes, GA-41 molecules intercalate into the region of the hydrocarbon chains of the phospholipids. At definite concentrations, GA-41 molecules induce the formation of certain defects in the structure of a bilayer, leading to the permeability of the liposomes for Pr^{3+} ions.

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