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Short Communication

ON THE TRANSPORT OF PESTICIDE LONTREL THROUGH LIPOSOMAL MEMBRANES

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Abstract: The process of ecotoxicant transfer through membrane was studied on the example of the pesticide lontrel (3,6-DCPA^{**}). 3,6-DCPA is able to penetrate through the model liposomal membrane. The mathematical model of mass-transfer process was established. It was found that the transfer rate of 3,6-DCPA through lipid membrane equals $(5-8) \cdot 10^{-10}$ M/s for all 3,6-DCPA concentrations.

Key Words: Lontrel, Liposome, Fluorescence, Mass-Transfer Rate.

INTRODUCTION

As regards the existing scale of pesticide application in agriculture and disposal of waste water and various technogenic derivatives into the environment, the development of methods of toxicological evaluation of the consequences resulting from these processes lags behind. The methods used for evaluation of dangerous pesticides or other toxicants are effort-, money-, and time-consuming and they do not provide exact data on the safety and long-term consequences of their application [1]. There arises the necessity to investigate biophysico-chemical principles of pesticide or action of other contaminants at the molecular level.

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** 3,6-DCPA, 3,6-dichloropicolinic acid; ϵ -ATP, etheno-derivative of adenosine triphosphorous acid (Na-salt).

So we carried out a series of biological, biochemical and physico-chemical studies on the interaction of a number of inorganic toxicants and pesticides and their combined action onto the key biological systems [2]. The investigations reveal some principal specific features in the ability of complex formation of pesticides with the most important components of cell, such as ATP, NADH, DNA.

The biological influence of xenobiotics involves a number of successive biophysical, biochemical, and chemical stages (xenobiotics transport through the biological membranes is the most important among them). In the present study the quantitative aspects of the process of transport of various natural contaminants through biological membrane were considered. The mass transfer processes were studied on the model system namely on liposome.

The most attractive property of liposomes is their closeness since they can be filled with various substances that are helpful for investigation of the ability of bimolecular layers to permeate ions and molecules. Liposomes are widely used in the studies of the effects produced by drugs, vitamins, hormones and antibiotics. We used liposomes for study of ecological toxicants ability to penetrate into cells. As an ecotoxicant the pesticide lontrel (3,6-DCPA) was investigated.

MATERIALS AND METHODS

The liposomes were produced from egg lecithin. Lecithin was used as commercial 10% ethanol solution having purity of no less than 98.5%. As in [3], lecithin solution at pH 7.2 with added ϵ -ATP and mixed with 10% of cholesterol was sonicated during 20 min at 0°C in argon flow (using ultrasonic disintegrator at operating conditions of 22 kHz and 0.4 A). The separation of free luminogen from encapsulated one was carried out by gel-filtration method on Sephadex G-50 under densitometer control. The sizes of obtained liposome were determined to be 60-100 nm according to [3].

An etheno-derivative of adenosine triphosphorous acid, ϵ -ATP (see Fig. 1a), was used as a fluorescent label with the excitation spectrum at 312 nm and with maximum fluorescence at 420 nm [4]. Fluorescence measurement was carried out with spectrofluorimeter "Aminco-Bowman" (USA) in 1-cm cuvette containing 2.7 ml of liposome solution in 0.05 M Tris-HCl buffer, pH 7.2. The concentration of encapsulated ϵ -ATP was equal to 10^{-4} M. The concentrations of introduced solution (0.3 ml) 3,6-DCPA were: 10^{-2} , 10^{-3} and 10^{-4} M. The fluorescence quenching was monitored for 19-21 hours. The experimental error did not exceed 10% in all series of tests.

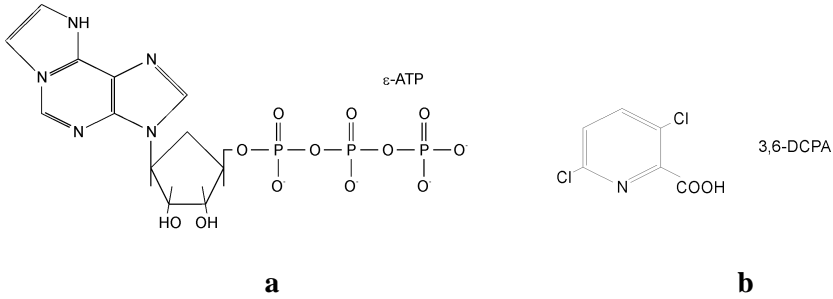


Fig.1. Chemical structures of luminogen (a) and quencher (b).

RESULTS AND DISCUSSION

In literature there are publications concerning fluorescence quenching of the labels incorporated into liposomes [5], however, ecotoxicants have never been used as quenchers. Earlier, we have found that pesticides comprise a new family of quenchers [6]. The data on the constant of ϵ -ATP - pesticide complexation [6] were used in the measurement of pesticide transfer into a liposome using label fluorescence quenching.

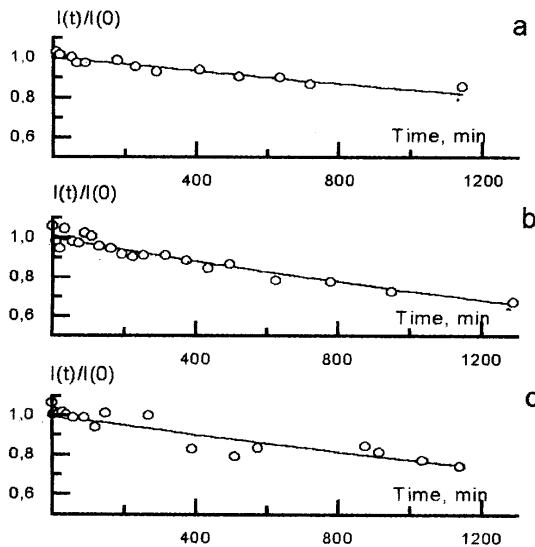


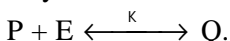
Fig 2. The dependence of the fluorescence intensity of the incorporated ϵ -ATP on the time of the 3,6-DCPA action. The concentrations of introduced solution (0.3 ml) of 3,6-DCPA were: a 10^{-4} M, b 10^{-3} M, c 10^{-2} M. [ϵ -ATP]= 10^{-4} M.

The experimental dependency of the fluorescence intensity I on the time of treatment with a quencher 3,6-DCPA (see Fig. 1b) is shown in Fig. 2.

It is seen that the ϵ -ATP fluorescence was quenched at considered concentrations of pesticide-quencher down to 70-60% of the initial intensity, i.e. complete quenching was not achieved.

As regards Fig. 2 the variation of introduced concentration of quencher within 10^{-4} - 10^{-2} M did not practically influence on the quenching curve.

The following simple model was considered to characterize quantitatively the lipid membrane permeability. The pesticide molecules P penetrate from solution having volume V_1 with the rate $j(t)$ into inside volume of liposome V_2 where they enter the reaction with the molecules of the ϵ -ATP denoted by E :



As a result of the reaction fluorescence quenching takes place. The system of equations describing the change of reagent concentration is as follows:

$$\frac{d([P] + [Q])}{dt} = j \quad (1)$$

$$K \cdot [P] \cdot [E] = [Q] \quad (2)$$

$$[Q] + [E] = [E_0] \quad (3)$$

$$[P_1] \cdot V_1 + ([P] + [Q]) \cdot V_2 = [P_0] \cdot V_0 \quad (4)$$

Here $[E_0]$ is initial concentration of the ϵ -ATP inside liposomes, $[P_1]$ and V_1 are concentration and the volume of the pesticide solution in the cuvette outside liposome, and P_0 and V_0 are the concentration and the volume of pesticide solution introduced into the cuvette. The equation (1) with the account of (2) and (3) may be transformed concerning the concentration $[E]$:

$$\frac{K \cdot [E]^2 + [E_0]}{K \cdot [E]^2} \cdot \frac{d[E]}{dt} = -j \quad (5)$$

The concentration of ϵ -ATP inside the liposome $[E]$ is connected with experimentally measured fluorescence intensity $I(t)$ by simple relationship $[E] = [E_0] \cdot I(t)/I(0)$, therefore the equation (5) can be used to determine the rate $j(t)$.

The values of the rates of pesticide transfer through liposome membrane have been found to depend weakly on pesticide concentration outside the liposomes and therefore on concentration gradient across the membrane. Respective values for pesticide concentration of 10^{-4} , 10^{-3} , and 10^{-2} M were determined to be

$(4.7 \pm 0.2) \cdot 10^{-10}$, $(8.7 \pm 0.2) \cdot 10^{-10}$, and $(6.8 \pm 0.2) \cdot 10^{-10}$ M/s, for which calculated quenching curves satisfactorily approximate experimental points. An average value of the rate of 3,6-DCPA transport through lecithin liposomal membrane is $(6.7 \pm 2) \cdot 10^{-10}$ M/s.

Thus, under considered conditions the rate of pesticide transport through lipid membrane is independent of concentration gradient and therefore seems to be determined by the properties of lipid bilayer and/or by pesticide molecule structure. The detailed mechanism of the pesticide transport through lipid bilayer may be found out in some additional studies, in particular involving pesticides of various structures.

In summary, our results lead to the following conclusions:

1. The pesticide Iontrel (3,6-DCPA) penetrates through the model liposomal membrane.
2. The pesticide flow through the membrane is independent of its concentration gradient across the membrane, i.e. it is determined by the membrane properties and/or by the toxicant molecule structure.
3. The rate of the mass-transfer of 3,6-DCPA through the liposomal membrane equals $(5-8) \cdot 10^{-10}$ M/s.

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