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AN INVESTIGATION OF THE MEMBRANOTROPIC PROPERTIES OF THE PLANT TOXIN THIONIN ISOLATED FROM *Pyrularia pubera*

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It has been shown by the ESR of spin probes that thionin initially interacts with with negatively charged membranes electrostatically and then passes into the membranes to a depth comparable with the length of the hydrophobic sections of the protein loops.

Thionin, isolated from the nuts of *Pyrularia pubera* Michx. (Santalaceae) [1] is a polypeptide consisting of 47 amino acid residues. The structure of the polypeptide is stabilized by four disulfide bonds, while high lysine and arginine contents make it strongly basic. Thionin belongs to the class of toxic polypeptides possessing hemolytic, cytotoxic, and neurotoxic actions [2]. The biological effect of action of thionin on cell membranes includes hemolysis of erythrocytes [2], inhibition of the growth of animal cells [3], activation of endogenous phospholipase A_2 of cell populations [4], and a number of other effects.

On the basis of the results of a previous investigation [5], it is assumed that the primary cause of the toxic effect of thionin is a change due to the polypeptide in the calcium permeability of cell membranes, with the subsequent triggering of biochemical reactions, such as the reactions of endogenous phospholipase, leading in the final account to the destruction of the membrane. It is not excluded that thionin, being a basic protein, may specifically bind with the acidic lipids of the membrane and perturb the initial packing of the phospholipid molecules, thereby disturbing the functioning of the membrane proteins and also the barrier properties of the lipid matrix.

The aim of the present work was to study the influence of thionin on the orientationally dynamic structure of the lipids of liposomes and the lipid matrix of erythrocyte membranes. One of the methods that is most sensitive to changes in the structure of a lipid matrix is the ESR of spin probes. As the spin probes we chose fatty acids bearing nitroxyl fragments at carbon atoms in different positions and the potential-sensitive probe CAT_{11} (Fig. 1).

Also using the ESR of spin probes we investigated the influence of thionin on liposomes formed from PC, but these experiments revealed no disturbances in the lipid matrix of the liposomes. In view of this, we selected model lipid systems consisting of mixtures of PC and acidic lipids. Moreover, the acidic lipids possess different molecular forms. Thus, CL and PC have the form of a reversed "wedge" that is capable of producing nonbilayer formations under appropriate conditions. These two facts induced to use in the present work mixtures of PC with various acidic lipids.

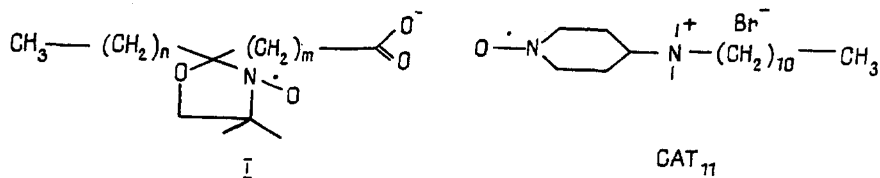

Fig. 1. Chemical structures of the spin probes used in this work.

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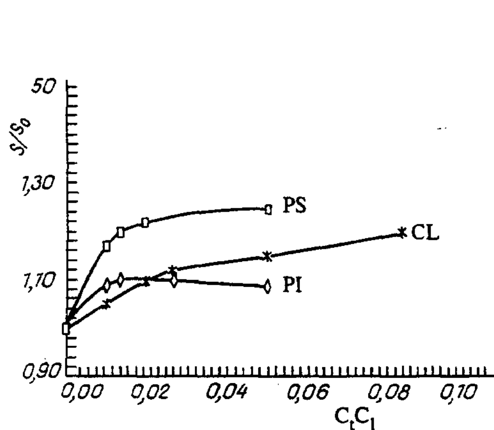

Fig. 2

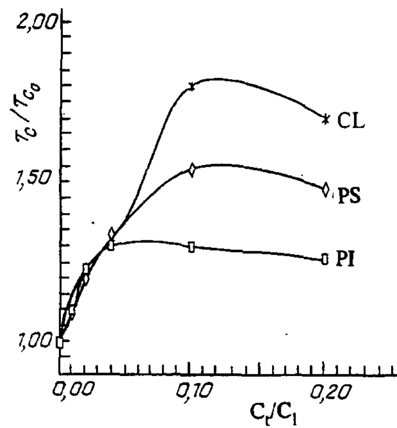

Fig. 3

Fig. 2. Dependence of the magnitude of the order parameter S of probe 5-DS on the concentration of thionin in lipid systems with various compositions.

Fig. 3. Dependence of the time of rotational correlation τ_c of probe 16-DS on the concentration of thionin in lipid systems with various compositions.

The experiments, which were conducted on liposomes formed from mixtures of PC with various acidic phospholipids (PC, CL, and PI), showed an appreciable immobilization of the nitroxyl fragments of probes 5-, 12-, and 16-DS. Thus, Fig. 2 shows the dependence of the change in the order parameter S of probe 5-DS, while Fig. 3 gives the change in the time of rotational correlation τ_c of probe 16-DS. The parameters S and τ_c evaluate the mobiity of the nitroxy fragment of a probe differently, and the corresponding clculations are made by different formulas. Consequently, there can be no complete correspondence between these parameters [7]. It can be seen that an increase in the concentration of thionin led to a disturbance of the initial structure of the lipid matrix. At low concentrations of thionin (up to S_t/S_{lip} = 0.04), "saturation" by it of the surface part of the monolayer, where the nitroxyl fragment of probe 5-DS is localized, took place, while higher concentrations (up to C_t/C_{lip} = 0.15) introduced more profound disturbances into the structure of the monolayer at the level of the nitroxyl radical of probe 16-DS. This permits the assumption that an increase in the concentration of thionin in the solution leads to an increase in the number of molecules of the peptide present within the bilayer, while the number of surface molecules does not change.

The change in the spectrum of probe 12-DS shows a change in the mobility of the nitroxyl fragment of the probe at the depth of the hydrophobic region of the bilayer in those sections where the binding of thionin molecules with phospholipid molecules takes place. Since the total ESR spectrum of the probe 12-doxylstearic acid is a superposition of the signal of two populations of probes with different mobilities, we may assume a separation of the lipid bilayer into sections with different dynamic mobilities of the phospholipid molecules. The greatest changes were undergone by the system containing CL, and the least by that with PS.

Measurement of the change in the parameter W of probe CAT_{11}, which is proportional to the surface charge of the membrane, showed that for the liposomes containing PS and PI there was a considerable decrease in the total negative charge of the surface of the bilayer with an increase in the proportion of thionin bound with it. This change in W was observed to a smaller degree in the case of CL molecules. This fact permits the assumption that in the first stage of the interaction of thionin with liposomes its molecules are bound by electrostatic interaction to sections of the bilayer bearing an excess negative charge. In the second stage there is a partial penetration of the hydrophobic sections of the loops of the chain of the toxin to a depth of the bilayer comparable with the length of these sections.

The investigations performed on model systems were repeated in experiments with biological membranes — erythrocyte membranes.

In samples of erythrocytes labeled with the probe 5-DS, an increase in the concentration of the toxin showed a smaller decrease in the value of $2T_{11}$ [7], which shows a weak disordering action of thionin in the hydrophobic region of the membrane

at the level of localization of the nitroxyl fragment of the probe. The spectral parameters of probes 12- and 16-DS, the nitroxyl fragments of which are located deeper in the hydrophobic region of the monolayer, scarcely changed with a rise in the concentration of thionin.

The reason for this surface perturbation by thionin molecules of the lipid composition of erythrocyte membranes may be connected with the fact that the bulk of the acidic lipids in erythrocytes is concentrated in the inner layer of the membrane, and, moreover, the thionin molecules enter into interaction not only with the lipid matrix but also with the protein components of the lipid membrane [5].

The results reported show that the thionin molecule interacts with acidic lipids of membranes, changing their structural organization.

EXPERIMENTAL

ESR spectra were recorded on a Bruker radiospectrometer (FRG) at a modulation amplitude not exceeding 20 mW. The radiospectrometer was fitted with a temperature attachment maintaining the given temperature in the resonator with an accuracy of ± 1°C. The error in the determination of the spectral parameters of the spin probes was not more than 10%.

The liposomes were formed from a mixture of egg phosphatidylcholine (PC) with phosphatidylserine (PS) (80 mole % of PC + 20 mole % of PS), with phosphatidylinositol (PI) (80 mole % of PC + 20 mole % of PI), and with cardiolipin (CL) (70 mole % of PC + 30 mole % of CL) as described in [6], the concentration of lipids in the experiments being 5 mg/ml.

As the spin probes we used 5- (m = 3, n = 12), 12- (m = 10, n = 5), and 16- (m = 14, n = 1) -doxylstearic acids (I) from Sigma and the probe CAT_{11}. The experiments were conducted with solutions of the probes in ethanol, which were added to the samples under investigation at a ratio of probe to lipid of 1:100.

Erythrocytes were obtained from donor erythrocyte material that had been washed with physiological solution and had been diluted with it to form a 2% cell suspension. Thionin was added from a buffer solution with a concentration of 1 mg/ml. For the preparation of the buffer solution we used Sigma Tris-HCl and EDTA. The other reagents were of ChKh ["chemically pure"] and ChDA ["pure for analysis"] grades.

REFERENCES

1. L. P. Vernon, G. E. Evett, R. D. Zeikus, and W. R. Gray, Arch. Biochem. Biophys., **283**, No. 1, 18 (1985).
2. G. E. Evett, D. M. Donaldson, and L. P. Vernon, Toxicon, **24**, No. 6, 622 (1986).
3. J. Evans, Y. Wang, K.-P. Shaw, and L. P. Vernon, Proc. Natl. Acad. Sci. USA, **86**, 5849 (1989).
4. C. K. Angerhofer, W. Y. Shier, and L. P. Vernon, Toxicon, **28**, No. 5, 547 (1990).
5. V. R. Ozorio e Castro and L. P. Vernon, Toxicon, **27**, No. 5, 511 (1989).
6. S. É. Gasanov, The Cytotoxin-induced Fusion of Phospholipid Membranes [in Russian], Dissertation for Candidate of Biological Sciences, Tashkent (1988).
7. A. N. Kuznetsova, The Spin Probe Method [in Russian], Moscow (1976).