SUMMARY

The polypeptides of regular structure $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$ modeling the terminal sections of various histones enriched with alanine and basic amino acids, have been synthesized. Analysis of the CD spectra of both polypeptides has shown that a tendency to the formation of the ELH conformation is characteristic for them, and this apparently is explained by the biological function of the terminal sections of histones which are responsible for their interaction with DNA.

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A STUDY OF THE INTERACTION OF THE CYTOTOXIN OF THE VENOM OF THE CENTRAL ASIAN COBRA WITH LIPOSOMES BY THE SPIN PROBE METHOD

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It has been shown by the spin probe method that the action of the cytotoxin of the venom of the central asian cobra on artifical liposomes extends at least to the depth of localization of the iminoxyl head of stearic acid spin-labeled at C-16. The greatest effect of the cytotoxin is observed on liposomes with a negative surface charge.

The cytotoxin (CT) of the venom of the cobra *Naja naja oxiana* forms a complex with natural and model phospholipid membranes through the interaction of the positive charges of the amino groups of the lysine residues with the negatively charged groups of the membranes and the hydrophobic sections immersed in them [1, 2]. However, the depth of the structural changes in the membranes caused by the action of CT is not clear.

In the present paper we consider the results of an investigation by the spin probe method of the structural changes in the hydrophobic region of liposomes caused by the action of CT. As the spin probe we used stearic acid spin-labeled at C-16 (Fig. 1). After its introduction into the membrane, the probe is orientated with its fatty chain parallel to the tails of the phospholipids of the membrane [3]. Consequently, a change in the electron spin resonance (ESR) spectrum of the probe will indicate changes in the membrane in the region in which its iminoxyl fragment is localized. For a quantitative estimate of the dependence of the structural changes in the membrane taking place under the action of CT on the surface charge of the membrane, we used liposomes prepared from neutral phophatidylcholine (PC), a mixture of the negatively charged phosphatidic acid (PA) and PC in a ratio by weight of 1:1, and also sonicated suspensions of PC which, together with liposomes, form lamellar structures.

The ESR spectrum of the probe in ethanol consists of a triplet signal with narrow components (Fig. 2, curve 1). When the probe is added to samples of suspensions of liposomes,

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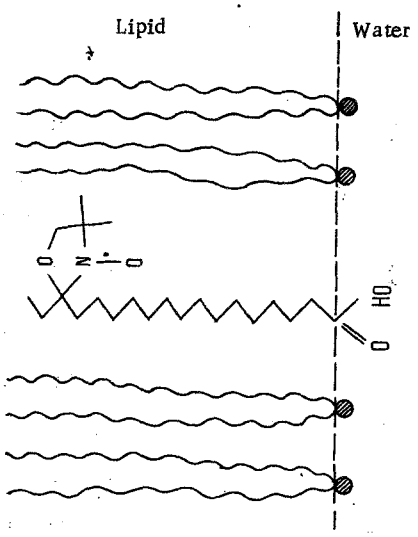

Fig. 1. Sketch of the arrangement of the spin probe in a liposome membrane.

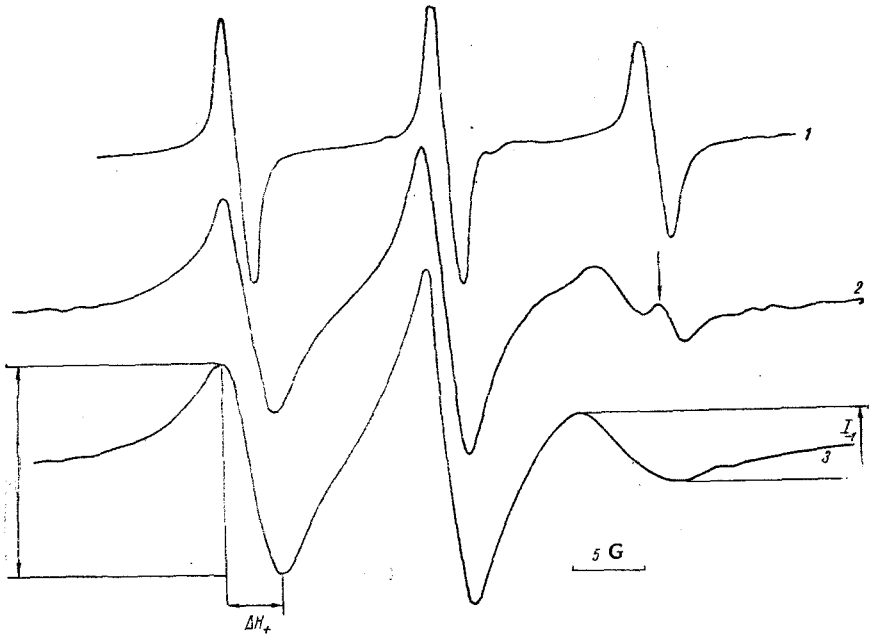

Fig. 2. ESR spectrum of the probe: 1) in ethanolic solution; 2) in suspension of liposomes without potassium ferricyanide (the arrow shows the component of the signal of the probe localized in the aqueous phase); 3) in suspensions of liposomes with potassium ferricyanide (10^{-2} M).

the ESR spectrum changes substantially and becomes a superposition of the signals from the probes that have penetrated into the membrane and those present in the aqueous phase (Fig. 2, curve 2). To separate the signals from the probe localized in the hydrophobic and hydrophilic regions, we used a method broadening the ESR spectra [4]. On contact with paramagnetic centers, the broadening agent, potassium ferricyanide, $K_3[Fe(CN)_6]$, broadens their spectrum and the signals from such paramagnetic centers are not recorded. When potassium ferricyanide was added to a suspension of liposomes, the signals of the probes localized in the aqueous phase disappeared, while the signals of the probes that had penetrated the membrane did not change (Fig. 2, curve 3). The ESR spectrum of the probe in a membrane has the form that is characteristic for probes with hindered motion [5]. For a quantitative estimation of mobility of the probe we used the correlation time τ_c [5].

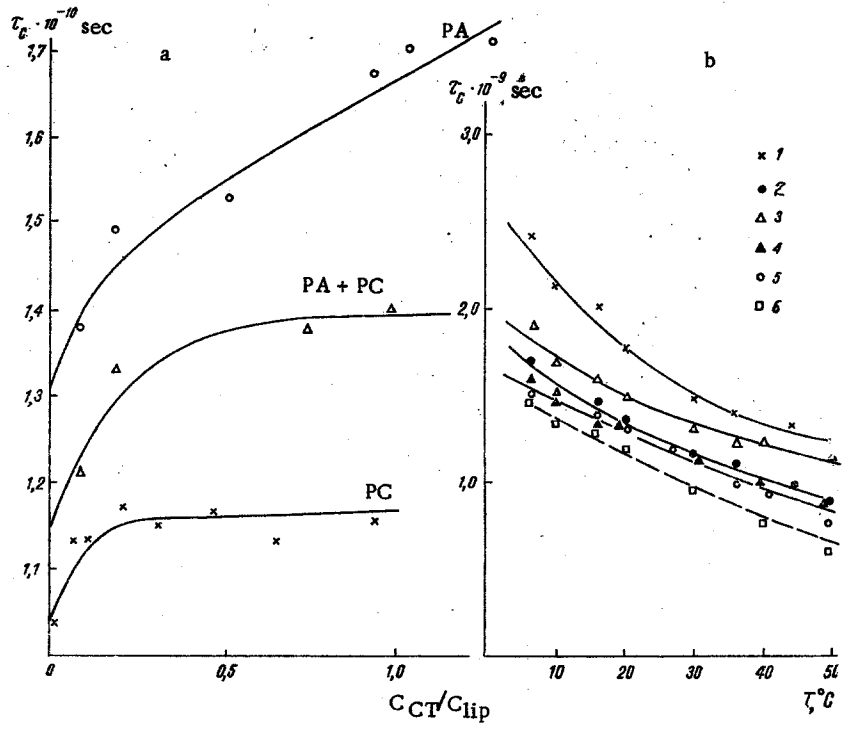

Fig. 3. Dependence of the correlation time τ_c on the concentration of CT in various samples (a) and on the temperature of the samples (b): 1) PA with CT; 2) PA without CT; 3) PA + PC with CT; 4) PA + PC without CT; 5) PC with CT; 6) PC without CT.

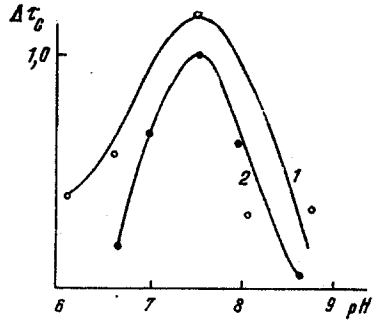

Fig. 4. Dependence of $\Delta\tau_c = \tau_c^{CT} - \tau_c$ on the pH (τ_c^{CT} with CT; τ_c without CT) for samples: 1) from PA; 2) from PA + PC.

The addition of CT to the samples of investigated liposomes led to a further hindrance to the motion of the probe, which, in its turn increased the correlation time τ_c. Figure 3a, shows the dependence of the parameter τ_c of the probe in various samples of liposomes on the concentration of CT. The greatest increase in the parameter τ_c is observed for samples obtained from PA and the least with those from PC, while, in the case of the mixed liposomes and samples from PC at certain concentrations of CT in the samples, τ_c reaches a maximum value and does not change with a further increase in the concentration of CT. For probes localized in samples derived from PA, τ_c does not issue onto a plateau in the range of concentrations of CT considered. An analysis of the results obtained shows that CT immersed in the membrane limits the mobility of the fatty chains of the phospholipids, and this action of CT extends to the depth of localization of the iminoxyl fragment of the probe. The greatest hindrance to the probe which, in all probability, is connected with a densification

of the structure of the membrane, is observed in samples prepared from PA, which have a fixed negative surface charge.

It is known [1] that CT interacts with biological membranes most effectively in the region of pH $\approx$ 7.5. Figure 4 shows the dependence of the correlation time τ_c on the pH of samples of a suspension of liposomes prepared from PA. As was to be expected, the greatest hindrance to the probe and, consequently, the maximum densification of the structure of the liposomes was recorded at pH $\approx$ 7.5.

We have also investigated the mobility of the fatty chains of the phospholipids of liposomes with CT and without it as a function of the temperature of the samples. With an increase in the temperature of the samples investigated there was a gradual "loosening" of the phospholipid layers, which was shown in a monotonic decrease in the correlation time τ_c of the probe (Fig. 3b).

EXPERIMENTAL

As the lipids we used phosphatidylcholine and phosphatidic acid obtained by known methods [6, 7].

The samples were prepared in the following way. A solution of PC or PA in chloroform was carefully dried, and then a buffer solution (Tris-HCl, pH 7.5, 10 mM) was added. A suspension of lipids with a concentration of 5 mg/ml was sonicated at 0°C in a UZDN-1 ultrasonic disperser at a frequency of 22 kHz for 25 min, and it was then neutralized in a TsLR-1 with an 18 × 0.01 attachment. Mixed PA + PC (in a weight ration of 1:1) liposomes were obtained by drying a chloroform mixture of PA and PC, followed by the procedure described above.

The cytotoxin was isolated from the venom of the central Asian cobra as described by Yukel'son et al. [8]. A solution of CT V''_c – 5 (40 mg/ml) was prepared in buffer solution (Tris-HCl, pH 7.5, ~10 mM) immediately before an experiment.

The spin probe was kindly supplied by É. K. Ruuge. An ethanolic solution of the probe was added to a suspension of liposomes to a final concentration of 10^{-4} M.

ESR spectra were taken on a Varian E-4 spectrometer, fitted with a temperature attachment, at a modulation amplitude not greater than 1 G and a power fed to the resonator not greater than 20 mW.

The correlation time τ_c was found from the formula

$$\tau_c = 6{,}65\left(\sqrt{\frac{I_{+1}}{I_{-1}}}-1\right)\Delta H_+ \cdot 10^{-10}\ \text{c}\ [5],$$

where I_{+1} and I_{-1} are the amplitudes of the low-field and high-field components of the spectrum; and ΔH_+ is the width of the low-field component determined as shown in Fig. 2, curve 3.

SUMMARY

The cytotoxin, on interacting with artificial liposomes, is immersed in the hydrophobic region. The change in the structure of the liposomes under the action of the CT extends into the depth of the phospholipid layers to at least the region of localization of the iminoxyl fragment of the spin probe.

CT acts most effectively on liposomes with a negative surface charge.

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A STUDY OF THE GLOBULIN OF COTTONSEEDS.

XXIV. ISOLATION AND CHARACTERIZATION OF A GLYCOPEPTIDE OF THE 7S GLOBULIN

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From a pronase hydrolysate of the 7S globulin of cottonseeds, using a column containing Sephadex G-25 and paper chromatography, a glycopeptide has been isolated which contains in its molecule residues of aspartic acid, glucosamine, and mannose in a ratio of 1:2:5. It has been shown by an analysis of the products of the methylation of the glycopeptide that the carbohydrate chain has a branched structure.

In an investigation of the structure of the 7S globulin of cottonseeds we have shown that the Asn-129 residue of the polypeptide chain of one of the subunits of the protein is bound to an oligosaccharide chain consisting of glucosamine and mannose [1]. In order to study the structure of the carbohydrate moiety, the protein was hydrolyzed with pronase and the glycopeptide was isolated by ion-exchange chromatography on Dowex 50 × 2 cation-exchange resin, gel filtration on Sephadex G-25 (Fig. 1), and paper chromatography. An individual glycopeptide was obtained during a study of the amino acid and carbohydrate composition, which revealed the presence of residues of aspartic acid, glucosamine, and mannose in a ratio of 1:2:5. It is obvious that the 7S globulin is a glycoprotein with a N-acetylglucosaminylasparagine binding group [2-4]. The presence of acetyl groups in the glucosamine residue was shown by the appearance of the signals of protons at 1.88-1.96 ppm, while the PMR spectrum of the glycopeptide obtained was similar to that of soybean agglutinin [2] (Fig. 2).

To establish the structure of the oligosaccharide we used Hakomori methylation after N-acetylation, followed by separation of the permethylate by lipophilic gel filtration on Sephadex LH-20 [5-7]. In the investigation of the hydrolysate of the permethylate by TLC, three products were obtained, two of which (with R_f 0.41 and 0.66) were identified as 2,3,6-trimethyl- and 2,3,4,6-tetramethylmannoses in comparison with standard substances. The third product (R_f 0.24) was assigned to the dimethylmannoses. The results obtained, when account was taken of the intensities of coloration of all three derivatives, permitted the assumption that the carbohydrate chain has branching at a mannose residue.

Thus, the oligosaccharide of the 7S globulin of cottonseeds has a branched structure similar to other plant and animal glycoproteins with N-glycoside type of carbohydrate–protein bond.

EXPERIMENTAL

Isolation of the Glycopeptide. The protein (5 g) in 200 ml of 0.05 M phosphate buffer, pH 7.8, containing 0.3 M sodium chloride and $1.5 \cdot 10^{-3}$ M calcium chloride, was hydrolyzed with pronase (Merck). Incubation was carried out for 20 h at an enzyme:substrate ratio of 1:100, and then new portions of the enzyme (1:50) were added at incubation times of 45 and 24 h. The resulting mixture was centrifuged, and the supernatant was lyophilized. The product was dissolved in 50 ml of acetic acid solution with pH 3.25, and it was passed through

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