We have studied the possibility of obtaining purified isoenzyme I (ISE-I) of PL-C from Cl. perfringens using affinity chromatography on polikefamid ["polycephamide"]. This sorbent has proved effective in the purification of phospholipases A_2 and D and also of lipases from various sources [1]. As the initial material we used a preparation of ISE-I from the culture liquid of the microorganism Cl. perfringens, strin VRGK No. 28, type A. The phospholipase activity in μmole of product in 1 min per 1 mg of protein was determined titrimetrically [2]. Affinity chromatography was carried out in the following way: 100 mg of initial preparation was dissolved in 0.05 M universal buffer (pH 5.6) containing 1 mM $ZnCl_2$ and was deposited on a column (1.5 × 2 cm) containing 200 mg of polikefamid equilibrated with the same buffer solution. After the elimination of ballast substances, the enzyme was eluted with sodium deoxycholate with a gradient change in the concentration of the latter from 0 to 1% in tris-HCl buffer (pH 7.0, 0.05) in the presence of 15% of glycerol. In spite of the high degree of purification of the ISE-I at this stage (64,800 times in comparison with primary concentrate from the culture liquid), an electrophoretic investigation in PAAG with sodium dodecyl sulfate showed the presence of ballast proteins in the material. Gel filtration was carried out for the additional purification of the ISE-I. The active fractions obtained after affinity chromatography were combined and concentrated to 2 ml with the aid of dry Sephadex-10. The concentrated protein was deposited on a column (1.6 × 72 cm) with Ultragel AcA-54 in 0.05 M phosphate buffer, pH 8.0, containing 0.1 M KCl, and the column was washed with the initial buffer. On electrophoresis, the preparation at this stage gave a single band. The degree of purification of the ISE-I amounted to 74,400 times at a yield of 38% on the activity of the initial preparation.

Chromatographic patterns in the purification of ISE-I are given in Fig. 1.

Thus, the ISE-I from the Cl. perfringens PL-C was obtained in a highly purified form by the method developed and, according to the results of disk electrophoresis in PAAG, was homogeneous.

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HEAT DENATURATION OF GOSSYPULIN (11S) FROM COTTON SEEDS. STUDY BY CD SPECTROSCOPY

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UDC 547.962.5

The thermal stability of proteins is an object of study by conformational physics [1-4] and is of interest for the physics of protein molecules and for the study of foods where the investigations performed have a direct issue into practice [5, 6].

Gossypulin (11S globulin) is the main reserve protein of cotton seeds. The properties of this protein largely determine the functional properties of cottonseed food protein. In salt solutions, gossypulin is present as three multiplet forms [7]. When the concentration of NaCl is lowered to less than 1%, the solubility of the protein decreases considerably [8]. It is well known that the heat stability of globulins depends on the concentration of salts in solution [9, 10]. For cottonseed globulin, this has been expressed in the difference of the regions of isoelectric precipitation of the protein after its heat denaturation in the absence and in the presence of salts [11]. In view of this, it appeared of interest to study the denaturation and renaturation of glossypulin in a 10% NaCl solution, pH 7.4 (at this pH value the protein has its maximum solubility in 10% NaCl solution).

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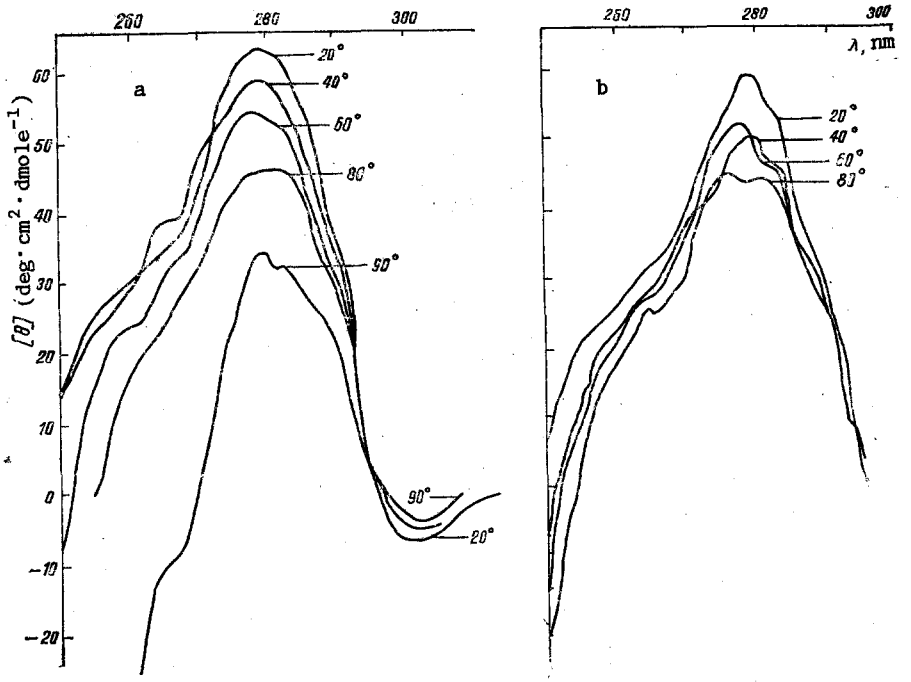

Fig. 1. CD spectra of the heat denaturation (a) and renaturation (b) of gossypulin in 0.05 M PBS, 10% NaCl, pH 7.4 (region of absorption of the aromatic system).

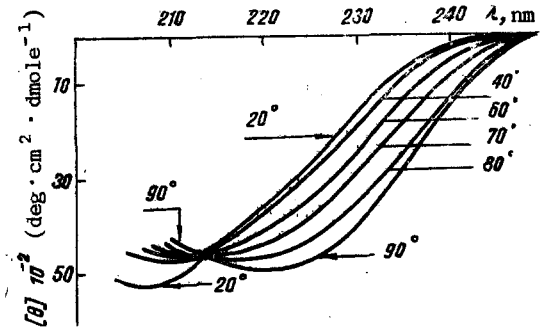

Fig. 2. CD spectra of the heat denaturation of gossypulin in 0.05 M PBS, 10% NaCl, pH 7.4 (region of absorption of the peptide bond).

The investigation was carried out by the method of CD spectroscopy [12]. Thermostated cells with jackets in which distilled water circulated at a given temperature were used. The temperature was maintained with the aid of a U-2 thermostat (GDR), and the rate of heating was 1 deg/min. After the necessary temperature had been reached, the sample was kept at it for 15 min and then the CD spectrum was recorded. Figure 1a shows the CD curves in the near ultraviolet. With a rise in the temperature to +80°C, no appreciable change in the value of the molar ellipticity at λ = 280 nm, $[\theta]_{\lambda_{280}}$, nor any displacement of the main bands at the maximum of the Cotton effect (CE) were observed. At +90°C, a distorted CD spectrum was obtained, since at this temperature the considerable aggregation of the protein led to a turbidity of the solution.

The change in the CD spectra in the far UV region was somewhat unusual (Fig. 2). The bathochromic shift of the CE band is apparently due to the contribution of the aromatic amino acids in the region of absorption of peptide bonds [13]. However, it must be mentioned that no changes in the value of the molar ellipticity due to a cooperative transition (melting of the protein globule) took place. The high thermal stability of the gossypulin structure in the presence of NaCl is also shown by the CD spectra of the products of the renaturation of the protein. As can be seen from Fig. 1b, the value of $[\theta]_{\lambda_{280}}$ increased when a solution

that had previously been heated to +80°C cooled. The CD curves of the initial sample (+20°C) and of the sample previously heated to +80°C were close.

Thus, gossypulin exhibits a high thermal stability in 10% NaCl solution which, in our view, is a consequence of the pronounced hydrophilic interactions between the hexamers of the protein [7]. This may also be the reason for the increased tendency to undergo aggregation at a high temperature in the presence of salts [11], which leads to an extension of the interval of the isoelectric precipitation of the protein in the alkaline region.

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SYNTHESIS OF THE γ-OCTADECYLAMIDE OF N-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE

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It is known that lipophilic derivatives of N-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyl dipeptide; MDP) usually possess a pronounced adjuvant and antitumoral action [1]. In order to study the influence of the method and position of introduction of a lipophilic component into the MDP molecule on its biological activity we previously obtained the heptyl and hexadecyl β-glycosides of the muramoyl dipeptide [2]. In the present communication we consider the synthesis of the γ-octadecylamide of N-acetylmuramoyl-L-alanyl-D-isoglutamine (I) – a lipophilic analog of MDP at the dipeptide fragment. In known methods of obtaining lipophilic derivatives of the muramoyl dipeptide at the carboxy group of the isoglutamine residue, modification is performed at the stages of obtaining the dipeptide [3]. We proposed to introduce the lipophilic fragment into the molecule of the protected glycopeptide at the free carboxy group of isoglutamine. In this way, it is possible to obtain a series of modifications, including lipophilic modifications, from a single glycopeptide.

The catalytic hydrogenolysis of the γ-benzyl ester of O-(benzyl 2-acetamido-4,6-O-isopropylidene-2-deoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine [4] over 10% Pd/C for 1 h gave a quantitative yield of O-(benzyl 2-acetamido-4,6-O-isopropylidene-2-deoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (II); $[\alpha]_{546}^{20}$ + 62° (c 0.69; chloroform); IR (cm^{-1}, KBr): 3380-3300 (OH, NH_2, NH); 1650, 1520 (amide), 850 (Me_2C); 730 (phenyl); PMR (500 MHz, MeOH-d_4): 1.36 d, 1.39 d (6H, $J_{CH_3,CH}$ = 7 Hz; $2CH_3CH$), 1.42 s, 1.50 s (6H; Me_2C), 1.91 s (3H; NAc), 2.32 t (2H, γCH_2), 4.51 d, 4.73 d (2H, J_{gem} = 12 Hz; OCH_2Ph), 7.02 s, 7.53 s (2H; $CONH_2$), 7.27-7.39 m (5H; Ph), 7.87 d, 8.24 d, 8.28 d (3H; 3NH). The N-hydroxysuccinimide ester of the acid (II), synthesized with the aid of N-hydroxysuccinimide and dicyclo-

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