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PURIFICATION OF ISOENZYME I OF PHOSPHOLIPASE C FROM Clostridium perfringens

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Phospholipases, including phospholipase C (PL-C, EC 3.1.4.3), are widely used in investigations of lipid metabolism and its disturbances and of the mechanisms of heterogeneous catalysis and in the analysis and chemcial synthesis of lipids, and are irreplaceable in the study of the structure and functions of biological membrane. The directions mentioned impose high demands on the purity of the preparations used. However, the majority of known methods of isolating and purifying PL-C from various sources cannot be regarded as satisfactory because of their low yield and the multistage nature of the processes.

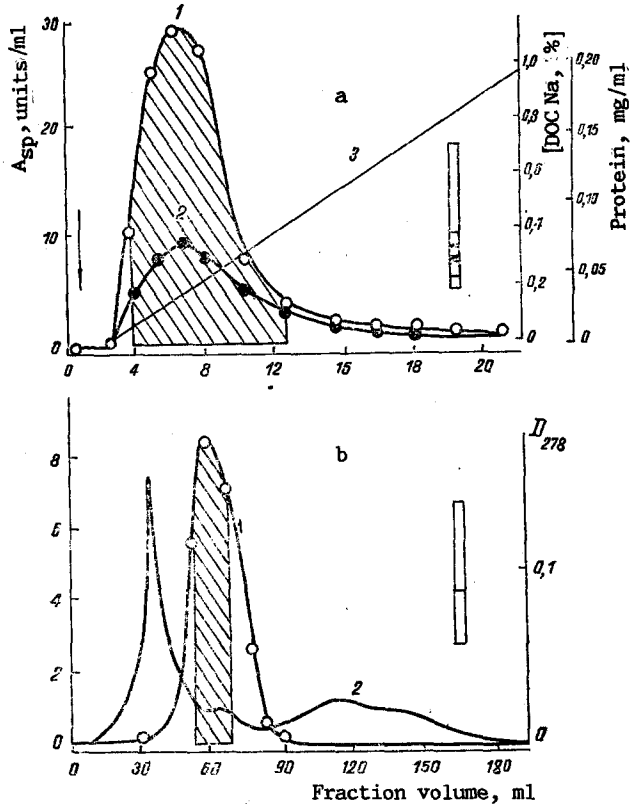

Fig. 1. Purification of isoenzyme I of phospholipase C from Cl. perfringens. a. Affinity chromatography on polikefamid: 1) activity; 2) protein; 3) sodium deoxycholate (arrow – beginning of elution). b) Gel filtration of AcA-54. The hatched region shows the fractions that were combined.

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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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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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