ISOLATION OF LYSOPHOSPHOLIPASE BY BIOSPECIFIC CHROMATOGRAPHY

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Lysophospholipases, together with phospholipases, are natural components of snakes and insect venoms that are responsible for their biological activity [1, 2]. We have isolated an enzyme with lysophospholipase activity from the venom of the giant hornet with the aid of gel filtration and ion-exchange chromatography on CM-cellulose [3]. However, because of the multistage nature of the process the yield of this enzyme was very low (about 1% in terms of protein and 6% in terms of activity) and we have therefore synthesized a special sorbent for the biospecific chromatography of the lysophospholipase. The sorbent was synthesized by the same procedure and was used for the synthesized of polyceramide — a group biospecific sorbent based on a polyamide proposed previously for the affinity chromatography of phospholipase from various sources [4, 5], with the only difference that as the ligand we used lysophosphatidylethanolamine. Egg yolk lysophospholipids were obtained by splitting the total phospholipids with phospholipase A_2 from *Naja naja oxiana* venom [6]. The degree of hydrolysis and the purity of the products were checked with the aid of TLC on Silufol UV-254 in the solvent system chloroform—methanol—water (65:25:4). The activity of the enzyme was determined by a titrimetric method on a T-108 pH-stat [7]. The specific activity of the enzyme was expressed in μmole of fatty acids liberated in 1 min (1 unit) calculated to 1 mg of protein.

The lysophospholipase from giant hornet venom was purified in the following way: 5 mg of the venom was dissolved in 2.5 ml of 0.01 M Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl, and the solution was deposited on a column (10 × 40 mm) containing 1 g, dry weight, of adsorbent equilibrated with the same buffer solution. The rate of adsorption and of the following operations was 12 ml/min, at a temperature of 20°C. After the elimination of ballast substances, the bound proteins were desorbed with ammonia solution having pH 11. After the active fractions had been combined, the pH of the solution was brought to neutrality, and it was subjected to lyophilization. The purity of the purified fractions was evaluated by disk electrophoresis in PAAG in the presence of Na SDS [8].

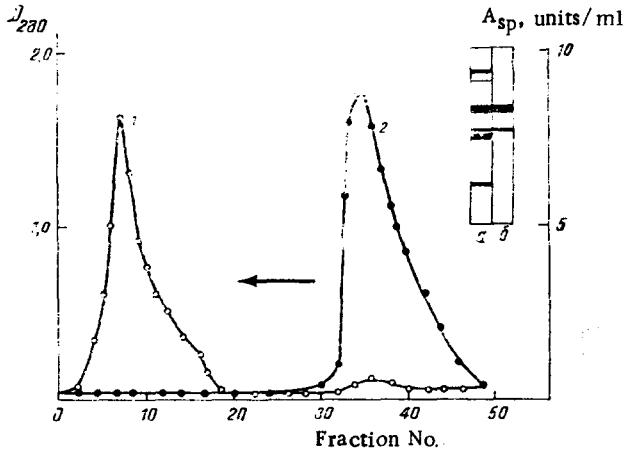

Fig. 1. Purification of lysophospholipase A_1 from giant hornet venom on polylysoceramide: D_{280} — optical density at 280 nm; A_{sp} — specific activity, units/ml; 1) protein; 2) activity. The arrow shows the beginning of elution by a 0.05 M Tris-HCl solution brought with ammonia to pH 11. The insert shows the results of electrophoresis before (a) and after (b) biospecific chromatography.

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The results of the biospecific chromatography of the giant hornet venom are shown in Figure 1. It can be seen that the bulk of the protein substances, possessing no lysophospholipipase activity, passed through the column without being bound to the adsorbent. When the column was washed with a solution of ammonia having pH 11, about 16% of the total activity of the initial venom was eluted. At this stage, the specific activity of the enzyme increased 5-fold. The noneluted part of the lysophospholipase (i.e., the immobilized form of the enzyme) also cleaved lysolecithin with a specific activity of 35-50 units per 1 g of sorbent. Analysis of the protein composition of the fraction eluted from the adsorbent showed two bands on an electrophoretogram with molecular weights of 32 and 44 kD. The gel filtration of this fraction on a column of Sephadex C-75 permitted the lysophospholipase to be separated from the accompanying component.

Thus, the lysophospholipase from giant hornet venom has been obtained in a highly purified form. An analysis of the terminal amino acids showed that phenylalanine was present at the N-end of the enzyme and lysine at the C-end which agrees with results obtained previously [9].

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INHIBITION OF THE OXIDATION OF LIGNIN BY SYNERGISTIC COMPOSITIONS

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As is known, the most effective inhibiting properties are possessed by systems in which there is a constant regeneration of the active forms of the inhibitors and which can act by several mechanisms: the inhibition of the formation of peroxides or their decomposition, the recombination of the inhibitor radicals with radicals of the substrate, or the disproportionation of the radicals formed. A substantial increase in the rate and selectivity of the alkaline delignification of wood is achieved with the use of anthraquinone [1], which forms reduced varieties: anthrasemiquinone and anthrahydroquinone [2].

We have established that the addition to anthraquinone of N,N-dimethyl-p-phenylenediamine under the conditions of an alkaline wood cook leads to the formation of a synergistic system, as is shown by an acceleration of delignification and a rise in the yield of cellulose by 2% as compared with an anthraquinone cook [3]. The possibility of increasing the antioxidant activity of the system by the addition of inhibitors of radical processes indicates a substantial contribution of redox transformations of the lignin in the wood-cooking process.

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