To create a density gradient we used sucrose, and, to a create a pH gradient, ampholite supports with a pH range of 3-5 (LKB). A density gradient of sucrose was created by mixing a light solution containing water and Ampholines and a heavy solution containing water, sucrose, and Ampholines. The sample that was to be subjected to isoelectric focusing was added to the light component.

On the use of an analytical column, the heavy electrode solution contained distilled water (14 ml) and sucrose (12 mg). The anode was located at the bottom of the column. Electrofocusing was carried out for 48 h with a voltage at the beginning of the experiment of 500 V and at the end of the experiment one of 1200 V and current strengths of 5 and 2 mA, respectively.

The sedimentation analysis of the enzyme was carried out on a MOM-3170 analytical ultracentrifuge. When a solution of the protein with a concentration of 7 mg/ml in 0.05 M sodium phosphate buffer, pH 7.8, was sedimented at 56,000 rpm with an exposure interval of 10 min one symmetrical schlieren peak was observed.

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# COMBINED IMMOBILIZATION OF LYTIC AND PROTEOLYTIC ENZYMES


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Lytic enzymes immobilized together with proteinases (lytic enzymes complex of Act. recifensis var. liticus 2435 and lysozyme) with retention of 80-150% of the lytic activity have been obtained. The properties of the preparations obtained have been studied.


The broad possibilities of the use of lytic enzymes in medicine are based on their direct action on microorganisms, and also on their immunostimulating, antiphlogistic, antiallergic, analgesic, and thrombocytopoesis-stimulating actions [1, 2]. However, the instability of the enzyme preparations and their high cost have led to a search for methods of immobilizing them [3-7]. In view of the fact that lytic enzymes could supplement the spectrum of the necrolytic action of proteinases immobilized on dressing material, we have carried out their co-immobilization.

Earlier, in the immobilization of lysozyme on a polyacrylamide support and on derivatives of Silokhrom, the percentage retention of lytic activity determined from the lysis of


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TABLE 1. Immobilization of the LEC

| pH | Borax, % | Yield of lytic activity, % |
|---|---|---|
| 6.0 | 0.5 | 50,9 |
| 6,5 | 0,5 | 71,9 |
| 7,4 | 0,5 | 60,4 |
| 6,0 | 1,0 | 40,8 |
| 6,5 | 1,0 | 48,5 |
| 7,4 | 1,0 | 17,3 |
| 6,0 | 2,0 | 52,3 |
| 6,5 | 2,0 | 35,8 |
| 7,4 | 2,0 | 19,7 |

TABLE 2. Co-Immobilization of Lytic Enzymes and Metalloproteinases on Gauze with the Aid of PVAlc Cross-Linked with Borax

| Lytic enzyme | Amount of lytic enzyme, mg/md $^2$ | Amount of metalloproteinase, mg/md$^3$ | Ratio of proteinase protein to lytic enzyme protein | Yield of lytic activity, % |
|---|---|---|---|---|
| LEC | 20 | 128,3 | 10:1 | 139,7 |
| " | " | 64,1 | 5:1 | 90,3 |
| " | " | 25,7 | 2:1 | 84,4 |
| " | " | 12,8 | 1:1 | 46,4 |
| Lysozyme | 1,4 | 128,3 | 10:1 | 206,6 |
| " | " | 64,1 | 5:1 | 158,3 |
| " | " | 12,8 | 1:1 | 132,1 |
| " | " | 1,3 | 0,1:1 | 153,1 |
| " | " | 0,13 | 0,01:1 | 126,3 |
| " | " | 0,013 | 0,001:1 | 94 |
| " | " | $1,3 \cdot 10^{-3}$ | 0,0001:1 | 55 |

TABLE 3. Influence of Proteinases on Lytic Activity

| Proteinase | Lytic enzyme | Ratio of proteinase protein | Lytic activity, % of control |
|---|---|---|---|
| Elastoterase | Lytic enzyme complex | 2:1 | 137,1 |
| " | " | 4:1 | 151,3 |
| " | " | 12:1 | 183,5 |
| Metalloproteinase | " | 2:1 | 159,4 |
| Protosubtilin | " | 6:1 | 213,7 |
| Terrilitin | " | 13:1 | 107,0 |
| Elastoterase | Lysozyme | 1:1,6 | 201,1 |
| Metalloproteinase | " | 1:7,5 | 144,4 |

microbial cells was ~4% [3, 4], while on cellulose diacetate and polycaproamide it was 14 and 29%, respectively [5]. An immobilized lysozyme with retention of 40% of its activity and stable for 49 days has been obtained by the interaction of high-molecular-matrices activated with silane reagents [6].

We have achieved the immobilization of enzymes on dressing materials with the aid of polyvinyl alcohol (PVAlc) cross-linked with borax.

Table 1 gives the results of the immobilization of a lytic enzyme complex (LEC) as a function of the pH of immobilization and the concentration of borax. On the basis of the results given in Table 1 and in the light of the inadequate cross-linking of the PVAlc at a concentration of borax of 0.5%, we carried out the co-immobilization of lysozyme and the LEC proteinases at pH 6.5 with 1% of borax.

The results, which are given in Table 2, show that, depending on the ratio of lytic enzymes and proteinase, the retention of lytic activity may reach 150-200% of the initial activity, and the retention of proteolytic activity 50-70%.

It is just the combined immobilization of proteolytic and lytic enzymes that has led not merely to the complete retention of the lytic activity but even to its increase to 120-150%.

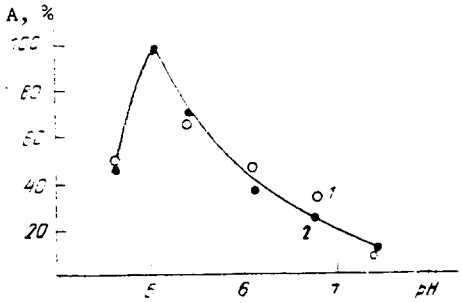


Fig. 1

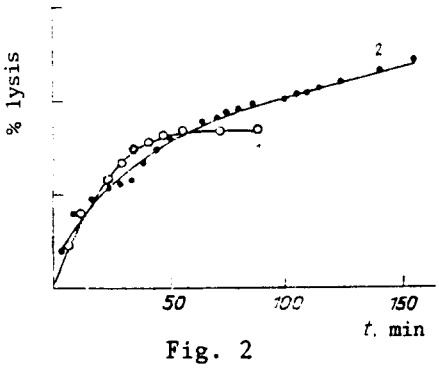


Fig. 2

Fig. 1. pH dependence of the activity of the native (1) and the immobilized (2) lytic enzyme complexes.

Fig. 2. Dependence of the degree of lysis of the cells of lactobacteria by the native (1) and immobilized (2) LECs on the time at 55°C.

A probable explanation of the immobilization results obtained is the activation of the lytic enzymes by the proteinases in this method of immobilization. The proteinases themselves exhibited no lytic activity. On our consideration of other methods of immobilization – for example, with the co-immobilization of proteolytic and lytic enzymes on carbonaceous materials – no increase in the activity of the lytic enzymes, was detected.

The effect of the increase in lytic activity on co-immobilization with proteolytic enzymes was observed with the metalloproteins elastoterase and protosubtilin, but was absent for terrilitin. Analogous results were obtained from the determination of lytic activity in solution in the presence of proteinases (Table 3). This is apparently connected with the specificity of the proteinases selected with respect to the peptide subunits of the peptidoglycan of bacterial cells.

The preparations that we had obtained possessed a considerably higher stability than lysozyme immobilized with the aid of silane reagents [6]. On storage for six months at 4-5°C they retained 95% of the initial lytic activity. The pH optima of the native and immobilized lytic enzyme complexes practically coincided (Fig. 1).

The study of heat inactivation of 55°C showed a greater stability of the immobilized lytic enzyme complex. The constants of thermal inactivation amounted to $1.7 \cdot 10^{-2}$ and $8.7 \cdot 10^{-3}$ $min^{-1}$ for the native and immobilized preparations, respectively. This probably also explains the greater stability of the immobilized lytic preparation than the native one during prolonged action on the substrate (Fig. 2).

## EXPERIMENTAL

The lytic enzyme complex from Act. recifensis var. liticus 2435, the metalloproteinase protosubtilin from the Ladyzhenka enzyme preparations factory of the Énzim Production Combine, elastoterase obtained in the Institute of Virology and Microbiology of the Ukrainian SSR Academy of Sciences, terrilitin from the L. Ya. Karpov Mosmedpreparaty Combine, and lysozyme produced by the Biolar Scientific Production Combine were used.

The lytic activities of the native and immobilized preparations were determined by the turbidimetric method from the optical densities of the incubation mixtures. A suspension of cells of Lactobacillus bulgaricus -51 with $D_{540}$ 0.6-0.7 and pH 6.0 and a sample volume of 4 ml was used as the substrate. As the unit of lytic activity we took that amount of enzyme that caused a fall in the optical density of the reaction mixture by 0.001 in 1 min at 55°C. The efficiency of immobilization was estimated in terms of the percentage retention of the initial lytic activity of the native preparation.

Proteolytic activities were determined by the method of Petrov and Vinugonaite [8]. As the unit of proteolytic activity we took the amount of enzyme that increased the absorption of the solution at 280 nm by 1.0 on incubation with casein for 10 min [9].

The amounts of protein in the preparation were determined by Lowry's method in Hartree's modification [10].

The pH dependence of lytic activity was determined by changing the pH of the incubation medium from 5 to 8.

The thermal stability of the lytic activity was determined after incubation of the preparations at 55°C and pH 6.0 for 5-90 min. The inactivation constants were calculated by means of the equation of a first-order reaction.

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# GLUTAMATE RECEPTOR BLOCKERS FROM VENOMS OF THE SPIDERS Argiope lobata AND Araneus tartaricus


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The presence of glutamate receptor blockers with molecular masses below 1000 Da and pI ≥ 9 has been detected in the venoms of the spiders Argiope lobata and Araneus tartaricus.


It has been found previously that the venom of the spider Argiope lobata blocks nerve-muscle transmission in insects and vertebrates by interacting with the structures of the postsynaptic membranes [1], while the effect on the glutamatergic synapses of the locust (Locusta migratoria) had an irreversible nature [1]. Kawai et al. [2] have reported that a low-molecular-mass toxin (JSTX) isolated from the venom of the spider Nephila clavata irreversibly blocks synaptic transmission in a nerve-muscle junction of the lobster. These investigations have served as a kind of impulse to the extension of the search for new glutamate receptor blockers. Thus, in recent years highly specific glutamate receptor blockers have been detected in an isolated from the venoms of various orb-weaving spiders (family Araneidae) [2-13]. It was found that they all — and at the present time they number more than ten — are, as a rule, low-molecular-mass components (<1000 Da) and have great structural homology [8, 12]. The basic structure of these molecules includes the amino acid arginine linked through a peptide bond with a polyamine which, in its turn, is linked to the carboxy group of asparagine the alpha-amino group of which is attached to a phenolic moiety


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