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COMPARATIVE STUDY OF THE HEMOLYTIC ACTION
OF TWO CYTOTOXINS FROM THE VENOM
OF THE CENTRAL ASIAN COBRA Naja oxiana Eichwald

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It has been shown that cytotoxins isolated from cobra venom differ in hemolytic activity; the greater activity of one of the cytotoxins corresponds to a more pronounced basicity of its molecules, which confirms the significance of a positive charge of a cytotoxin molecule in the mechanism of its lytic action.

Cytotoxins – basic polypeptides represented in the venoms of elapid snakes by numerous molecular forms – cause cell lysis which is aggravated by the addition of phospholipases A_2 . It is assumed that the lipid effect of the cytotoxins is due to their capacity for labilizing the structure of cytoplasmic membranes, the main role in this being assigned to the hydrophobic properties and pronounced positive charges of the cytotoxin molecules [1, 2]. Two cytotoxins, V_{C1} and V_{C5} have been isolated previously from the venom of the Central Asian cobra [3]. The amino acid sequences of the two cytotoxins were characterized by a high degree of homology [4, 5], but cytotoxin V_{C1} was distinguished by a smaller lysin content and a larger content of dicarboxylic amino acid residues, which corresponded to the lower basicity of its molecule found on electrophoresis [6, 7]. It appeared possible that the comparative functional analysis of the two cytotoxins would permit an estimation of the significance of the positive charges of their molecules in the mechanism of their action on membranes.

Direct hemolysis is the most commonly used method of evaluating the membrane activities of different lytic agents. The direct hemolytic activities of the cytotoxins that we were investigating depended on their concentration in the medium and the time of incubation with washed erythrocytes. In both variants of the experiments we found a fairly high similarity of the kinetics of the direct lytic effects of the cytotoxins being compared, but at the same time, cytotoxin V_{C5} was characterized by a higher hemolytic efficiency: the calculated HD_{50} values for cytotoxins V_{C1} and V_{C5} proved to be 2000 and 1400 μg , respectively. The lytic effects of both cytotoxins also showed similar dependences on the pH of the medium. With a rise in the absolute pH of the medium, when the ionization of the negatively charged groups of a membrane increases, the interaction of the cytotoxins with the membranes was intensified, the more basic cytotoxin V_{C5} lysing the erythrocytes more actively than cytotoxin V_{C1} . Characteristic for the temperature dependence of the lytic effect of the cytotoxins was an increase in the direct hemolytic effect with a rise in the temperature to 30°C, while with a further rise in the temperature of incubation the hemolytic effect of the cytotoxins fell sharply (Fig. 1). It is known that at physiological temperatures a cooperative structural rearrangement of membranes takes place which is accompanied by their labilization. The cytotoxins probably attack such destabilized membranes more vigorously.

We have shown previously that calcium ions inhibit the direct hemolytic effect of cytotoxin V_{C5} [2]. With an increase in the concentration of calcium ions in the medium, a fall is also observed in the direct hemolytic activity of cytotoxin V_{C1} while in the presence of phospholipase A_2 the addition of calcium ions considerably enhances the synergistic hemolytic effect of cytotoxin V_{C1} and the enzyme in a similar manner (Fig. 2) to what has been found previously for cytotoxin V_{C5} [2].

On the whole, the results of the investigations performed reveal a fairly high similarity in the hemolytic effects of the two cytotoxins. The higher hemolytic effect of cytotoxin V_{C5} observed in all cases correlates with the greater basicity of its molecules. This correlation gives a confirmation of the significance of the positive charge of cytotoxin molecules in the mechanism of their lytic action postulated previously on the basis of an analysis of their structures and interactions with synthetic phospholipid membranes [6, 8].

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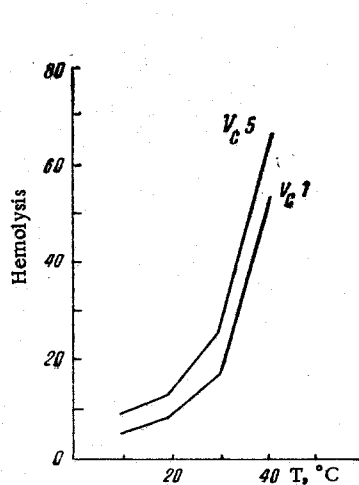


Fig. 1

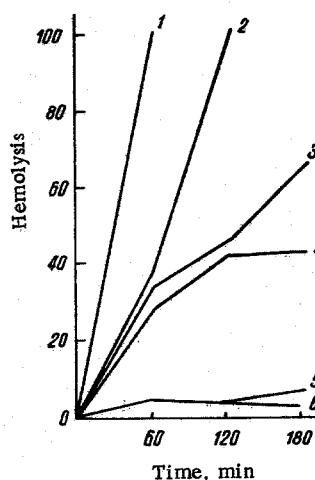


Fig. 2

Fig. 1. Temperature dependence of the direct hemolytic effects of cytotoxins V_{C1} and V_{C5} (400 μ g). Incubation medium (volume 0.2 ml): 50% suspension of washed human erythrocytes (0.05 ml) and solutions of the cytotoxins (0.15 ml).

Fig. 2. Influence of Ca^{2+} ions on the potential hemolysis caused by the combined action of cytotoxin V_{C1} (400 μ g) and phospholipase A_2 (50 μ g) (for the incubation medium, see Fig. 1): 1) cytotoxin, phospholipase A_2 , 5 mM Ca^{2+} ; 2) cytotoxin, phospholipase A_2 , 0.5 mM Ca^{2+} ; 3) cytotoxin, phospholipase A_2 ; 4) cytotoxin; 5) phospholipase A_2 , 5 mM Ca^{2+} ; 6) phospholipase A_2 , 0.5 mM Ca^{2+} .

EXPERIMENTAL

Pure preparations of the cytotoxins and of phospholipase A_2 were obtained from the venom of the Central Asian cobra *Naja oxiana* Eichwald by methods described previously [3, 6, 9]. Hemolytic activity was evaluated by known methods [10]. We used 50% suspensions of human erythrocytes washed three times with physiological solution and taken in an isotonic medium. The degree of hemolysis was expressed in percentages calculated from the liberation of hemoglobin, the amount of which was measured spectrophotometrically from its absorption at 540 nm.

SUMMARY

It has been shown that with a considerable overall similarity in the kinetics of the direct hemolytic effect cytotoxin V_{C5} is characterized by a greater hemolytic efficiency than V_{C1} . The high lytic activity of cytotoxin V_{C5} corresponds to the more pronounced basicity of its molecules. This correspondence confirms the significance of the positive charges of cytotoxin molecules in the mechanism of their lytic action.

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AMOUNTS OF PROTEIN AND OIL AND ACTIVITY OF THE TRYPSIN INHIBITOR IN DIFFERENT VARIETIES OF SOYBEAN

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Fifteen varieties of soybean have been investigated for the amounts of protein, oil, and trypsin inhibitor that they contain. A high thermal stability of the latter has been detected. Information is given on the kinetics of the inhibition of the amidase and proteinase activity of trypsin. It has been shown that the "trypsin inhibitor" consists of a mixture of six proteins.

Soybeans occupy an exceptional place among leguminous and cereal crops, since their protein is the only one out of all plant proteins that is close in food value to the proteins of animal origin [1]. It must be mentioned, however, that soybean seeds contain larger or smaller amounts of inhibitor proteins that are responsible in certain cases for reducing the assimilability of soybean protein by animals because of the inhibition of the proteolytic enzymes of the digestive tract and, in the first place, of trypsin and chymotrypsin [2-4]. Furthermore, it has been established that these inhibitor proteins are far from harmless. They not only suppress the growth of the animal but may also cause hypertrophy of the pancreas [5, 6]. Consequently, a real increase in the food value of soybeans may take place in two directions. The first is the freeing of the nutrient protein of the soybean from the accompanying inhibitor proteins in the process of preparing the beans, which is economically unfavorable, and the second is the isolation of new varieties of soybean with a reduced content of inhibitor proteins. In the latter case, these inhibitors become peculiar genetic markers that can be used both in the analysis of already existing genetic material of the soybean and in the process of isolating new varieties. In view of this, it must be stated that there is another important factor - proteins inhibiting proteolytic enzymes have an independent value, since a number of preparations used in medical practice have been created from them abroad. We have investigated 15 varieties of soybean grown in one of the regions where it is cultivated, Tadzhikistan, for their contents of oil and protein and have also made a comparative study of the activities of the inhibitor proteins in relation to trypsin. As can be seen from Table 1, the amounts of protein in different varieties of soybean range between 30.3 and 43.4%, and the amounts of oil between 14.3 and 28%. According to the figures in Table 1, the varieties of soybean investigated can be divided in relation to the protein and oil in the seeds into three groups: 1) the protein-rich, oil-rich varieties (1-5); 2) the protein-poor, oil-rich varieties (7-15); and 3) a protein-rich, oil-poor variety (6). The mean values of the ratios of the amounts of protein and oil in these groups are 1.87, 1.41, and 2.76, respectively, the sum of protein and oil ranging from 65.2 to 51.3%.

The specific activity of the trypsin inhibitors from the different varieties of soybean ranged from 17 to 135 units/ml of extract (determined in the presence of N-benzoyl-D,L-arginine p-nitroanilide (BAPA)) and from 1.95 to 11.5 units/ml of extract (determined with respect to casein). These results show the absence of a connection between an increased content of protein and oil and the specific inhibitor of the trypsin inhibitors isolated from soybean seeds.

It may be assumed that the difference in the activities of the trypsin inhibitors present in soybean protein depends on a genotypic feature of a particular variety. In view of this, the activity of the trypsin inhibitors may be a good test for selecting those varieties that would combine a high protein content with a low activity of the trypsin inhibitors. From this point of view, according to the figures in Table 1, the most promising varieties

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