SELECTIVITY OF THE MEMBRANE ACTION OF A CYTOTOXIN FROM THE VENOM OF THE CENTRAL ASIAN COBRA

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UDC 612.017.4:577.111/577.352.2

It has been shown that the selectivity of the direct hemolytic effect of the cytotoxin on different erythrocytes correlates with its influence on the ionic permeability of the membranes of these erythrocytes. It has been established on bilayer phospholipid membranes that the influence of the cytotoxin on their ionic permeability depends on the amount of cholesterol in them. It is assumed that the resistance of the cells to the cytotoxin is determined by the stability of the phospholipid backbone of their membranes.

Cytotoxins — polypeptides characterized by a high content of nonpolar and positively charged amino acid residues simultaneously — have been found in the venoms of various snakes of the Elapidae family [1, 2]. The biological effects of the cytotoxins are diverse, but their fundamental characteristic must be considered a direct lytic effect and the capacity for potentiating the lytic action of phospholipase A_2 [3]. In this process the cytotoxins exhibit selectivity with respect to various types of cells, and the potentiated action repeats the selectivity of the direct effect [4, 5]. In the present paper we consider the selective actions of a cytotoxin which we have isolated in the pure form from the venom of the Central Asian cobra.

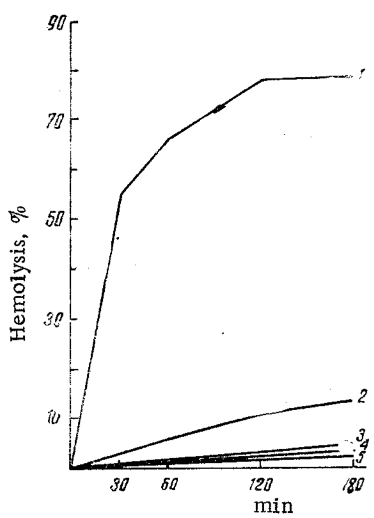

Fig. 1

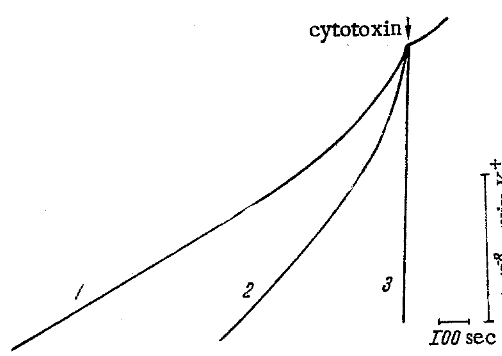

Fig. 2

Fig. 1. Direct hemolytic effect of the cytotoxin, v_c5 on erythrocytes of various species of animals. Incubation medium (0.2 ml): 0.05 ml of a 50% suspension of washed erythrocytes and 0.1 ml of an isotonic solution of sodium chloride containing 100 μg of the cytotoxin. Erythrocytes: 1) guinea pig; 2) man; 3) rat; 4) horse; 5) rabbit.

Fig. 2. Influence of the cytotoxin v_c5 $(1 \cdot 10^{-6}$ M) on the issuance of K^+ ions from erythrocytes of various species of animals. Suspensions of erythrocytes with $E_{750} = 0.065$ in isotonic sodium chloride solution, pH 7.4, were used. Erythrocytes: 1) rat; 2) rabbit; 3) guinea pig.

Institute of Biochemistry of the Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 813-817, November-December, 1980. Original article submitted June 30, 1980.

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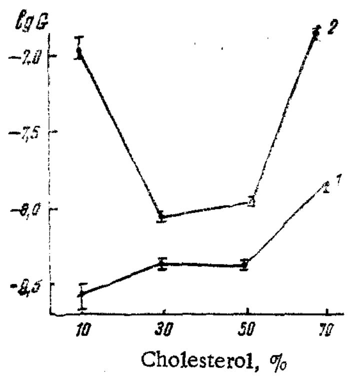

Fig. 3

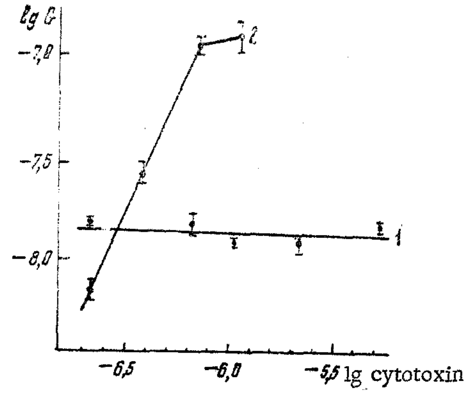

Fig. 4

Fig. 3. Action of the cytotoxin v_c5 on the conductivity of lecithin–cholesterol bilayer phospholipid membranes (for the experimental conditions, see text): 1) control; 2) experiment ($1 \cdot 10^{-6}$ M cytotoxin).

Fig. 4. Action of various concentrations of cytotoxin v_c5 on the conductivity of lecithin–cholesterol bilayer phospholipid membranes containing 50% (1) and 10% (2) of cholesterol (for the experimental conditions, see the text).

Investigations of the direct lytic action of the cytotoxin on various erythrocytes have revealed its greatest efficacy in relation to guinea pig erythrocytes; the other erythrocytes used in our experiments formed the sequence man–rat–horse–rabbit cells (Fig. 1). We have shown previously [6-9] that the cytotoxin increases the ionic permeability and decreases the stability of biological and artificial phospholipid membranes. An increase in the permeability of the membranes was considered as a functional sign of disturbances and labilization of the whole structure of the membrane caused by the cytotoxin. In view of this, it appeared of interest to investigate the influence of the cytotoxin on the permeabilities of the membranes of different erythrocytes. The results obtained (Fig. 2) show that in the presence of the cytotoxin the rate of issuance of K^+ ions from guinea pig erythrocytes is considerably higher than from rabbit and rat erythrocytes. Thus, the increase in the ionic permeability of the membranes in the presence of prelytic concentrations of the cytotoxin correlates with the selectivity of its lytic action on different erythrocytes.

Although the reasons for the selectivity of the lytic action of the cytotoxin on the various cells are unclear, it may be assumed they are due to features of the phospholipid composition of their membranes. In this connection important information is given by the ratio of the sphingomyelin and lecithin in the membranes [10, 11]. However, our experiments [12] in which we investigated the influence of the cytotoxin on the permeability of liposomes formed from pure sphingomyelin and the total phospholipids of human erythrocytes revealed no differences in the cytotoxin-induced rate of issuance of K^+ ions from them.

The cytotoxin also increased the conductivity of bilayer phospholipid membranes prepared from the total phospholipids of various erythrocytes and ox brain, and also from lecithin almost identically; the cytotoxin exhibited a considerably smaller effect only on membranes from oxidized cholesterol [12]. In view of this, the hypothesis was put forward that the amount of cholesterol, affecting the density of packing and the physical state of the phospholipids in the membrane, may be one of the factors determining the resistance of the cell membranes to the cytotoxin. Subsequently, we also investigated the action of the cytotoxin on the conductivity of bilayer phospholipid membranes characterized by different ratios of lecithin and cholesterol (Fig. 3).

The results obtained show that the conductivity of the control bilayer phospholipid membranes as a whole rises with an increase in the concentration of cholesterol. On the curve of the dependence of the conductivity of the bilayer phospholipid membranes on the concentration of cholesterol in a membrane-forming solution of phospholipid a "plateau" section is observed which corresponds to the conductivity of artificial membranes containing 30-50% of cholesterol, which agrees with literature information [13]. On the whole, the cytotoxin

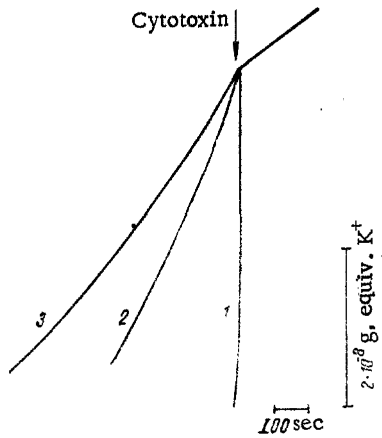

Fig. 5. Action of cytotoxin v_C5 ($2\cdot10^{-6}$ M) on the issuance of K^+ ions from control (1) and sonicated (2, 3) liposomes formed from the total phospholipids of human erythrocytes. The liposomes were sonicated in a UZDN-1 ultrasonic generator at 0.3 A and 35 kHz for 30 sec (2) and for 10 min (3).

(10^{-6} M) increases the conductivity of all bilayer phospholipid membranes, but those containing 10 and 70% of cholesterol to a greater extent.

The cytotoxin has a considerably smaller influence on the conductivity of membranes containing 30 and 50% of cholesterol. These results were confirmed in another variant of the experiment in which we investigated the action of various concentrations of the cytotoxin (from $2\cdot10^{-7}$ to $5\cdot10^{-6}$ M) on the conductivity of bilayer phospholipid membranes containing 10 and 50% of cholesterol (Fig. 4). Only in the first case was an increase in the conductivity of the artificial membrane with a rise in the concentration of cytotoxin observed; in the case of the membranes containing 50% of cholesterol, even high concentrations of toxin ($5\cdot10^{-6}$ M) caused no increase in conductivity.

The similarity of the effect of the cytotoxin on biological and artificial membranes [14] enables the results that we have obtained to be interpreted in connection with the general mechanism of its lytic action and its selectivity with respect to individual cells. In the mechanism of the action of the cytotoxin we ascribe the main role to the basic and hydrophobic properties of its molecules, assuming penetration of the cytotoxin electrostatically bound to the membrane into the hydrophobic zone of the membrane followed by the labilization of the structure of its phospholipid backbone. In this process, the limiting stage may be the phase of penetration of the cytotoxin into the membrane. Consequently, attention must be directed to the condensing and orienting effect of the cholesterol, thanks to which the structure of the phospholipid bilayer of the membrane is stabilized and its mechanical strength is raised [15]. The significance of the latter parameter can be judged from the results of our experiments in which we investigated the action of the cytotoxin on sonicated and unsonicated liposomes (Fig. 5). On sonication of the liposomes, accompanied by a decrease in their size and a rise in their mechanical strength, the issuance of K^+ ions from them caused by the cytotoxin was inhibited.

Thus, it appears possible that the cytotoxin acts more effectively on membranes with a smaller cholesterol content in which the obstacles to its effective penetration into the hydrophobic zone on the membrane have been eliminated. At a high cholesterol content when the structure of the membrane is labilized and is characterized by a fairly high permeability in the control, the cytotoxin again acts effectively. Less sensitive to the cytotoxin are membranes which are more stable as the result of definite ratios of cholesterol and phospholipids. However, all this excludes a possible role of the proteins of the membrane as additional factors determining the sensitivity of the cells to the cytotoxin.

EXPERIMENTAL

A pure preparation of the cytotoxin was isolated from the venom of the Central Asian cobra by the method described previously [2]. The purity of the lecithin, which was obtained from egg yolk [15], was checked by thin-layer chromatography in various solvent systems. Recrystallized cholesterol produced by the Leningrad Meat Combine was used. The total phospholipids of human erythrocytes were obtained by the method usually adopted [17]. Hemolytic activity was evaluated by the method of Aloof-Hirsch et al. [18]. The formation of the bilayer phospholipid membranes and liposomes, and also the study of the electrical parameters of the bilayer phospholipid membranes, were carried out by methods described previously [8, 12, 19]. Where necessary, the liposomes were sonicated in a UXDN-1 ultrasonic generator. The rate of issuance of K^+ ions was recorded with the aid of a selective valinomycin electrode by a pH 340 pH-meter with a recorder coupled to it.

SUMMARY

It has been established that the selectivity of the direct lytic effect of the cytotoxin on erythrocytes of various animals correlates with its influence on the ionic permeability of the membranes of these erythrocytes.

The influence of the cytotoxin on the conductivity of the bilayer phospholipids membrane depends on the amount of cholesterol in them.

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