PROTEOLYTIC ACTIVITY OF THE VENOM OF *Echis multisquamatus*

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The venom of the viper *Echis multisquamatus* is of interest as a possible source of a number of valuable biologically active substances used in scientific studies and in medicine. The venom of this viper, which is endemic for Near and Central Asia [1] has not previously been studied in detail. There have been few publications characterizing individual components of this venom [2, 3] and practically no information on the composition and properties of the proteolytic enzymes present in it. Nevertheless, many workers [4, 5] connect the various biological effects of snake venoms, including their lethal action and their influence on the blood-clotting reaction, with the activity of proteases.

The proteolytic activity of the venom of the Central Asia viper as judged from the hydrolysis of such protein substrates as hemoglobin (Reanal), casein produced by the Olaine factory – both intact and methylated by a known method [6] – kininogen obtained from blood serum, and synthetic bradykinin [7, 8]. The quantitative estimation of activity was made with the aid of generally adopted methods [8, 9]. In view of the fact that some proteinases are active in relation to esters of individual amino acids, we also investigated the esterase activity of the venom in the hydrolysis of benzoyl-L-arginine ethyl ester (BAEE) [8].

It was found that the hydrolysis by the venom of a protein (hemoglobin) took place ineffectively in acid media. The activity of the whole venom in relation to kininogen and bradykinin could not be evaluated quantitatively, either. On the other hand, the proteolytic activity of the venom in alkaline media, determined from the hydrolysis of casein, was high, and in the case of methylated casein amounted to 27 units (the units of specific activity

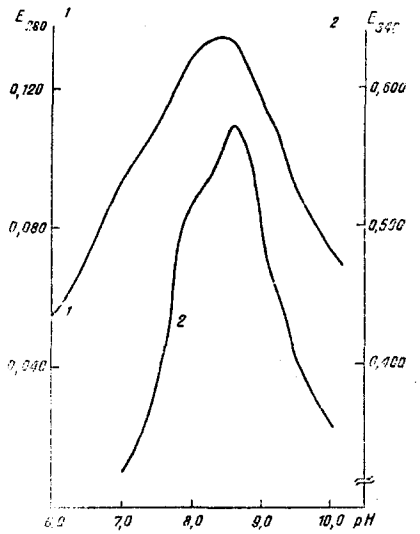

Fig. 1. pH dependence of the proteolytic activity of the viper venom on intact (1) and methylated (2) caseins. The concentrations of venom were 250 μg/0.1 ml (1) and 100 μg/0.1 ml (2).

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corresponded to the number of NH_2 groups liberated, which was calculated from the well-known formula [6]

$$NH_2 = \frac{A_{340}}{1.3 \times 10^4} \times 10^6,$$

where 1.3×10^4 is a recalculation factor; and

A_{340} is the absorption at a wavelength of 340 nm).

Figure 1 shows the pH dependence of the caseinolytic activity of the venom, showing a pH optimum of 8.6.

The whole venom also possessed a fairly powerful BAEE esterase activity, which was calculated as 7.7 units (the unit of specific activity corresponded to the amount of substrate hydrolyzed (in μmole) calculated from the formula [8]:

$$C = \frac{a \times 2.73}{b} \quad \mu \text{ mole of BAEE/mg/ml},$$

where C is the specific activity;

a is the increase in optical density; and

b is the amount of material in the sample, mg.

Thus, the results of our investigations have revealed in the venom a protease showing affinity with trypsin and they indicate a low activity of cathepsins and the complete absense of a kininogenase and a specific kininase. A high activity of trypsin-like proteinases is characteristic for the venoms of other snakes [4, 10]. The absence of other types of proteolytic activity may also be due to the presence in the venom of inhibitors similar to the inhibitor of carboxypeptidyldipeptidase which have identified in the venom of the Central Asian viper previously [3].

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