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ANALYSIS OF THE PHOSPHATASE ACTIVITIES OF THE VENOM OF RENARD'S VIPER BY ISOELECTRIC FOCUSING

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Snake venoms contain phosphomono- and phosphodiesterases with different degrees of substrate specificity. A clear idea of the composition of snake venoms is complicated by the existence of numerous forms of these enzymes. We have separated the venom of Renard's viper into fractions by isoelectric focusing and have analyzed the phosphatases in them.

When using ampholines creating a pH gradient from 3.5 to 9.5 it was possible to separate Renard's viper venom into 19 components. During the separation the following phosphatase activities were measured: those of nonspecific alkaline monophosphatase and of 5'-nucleotidase, determined by the method of Dixon and Purdom [1], cited by Orlowski [2], from the splitting off from the substrate of inorganic phsophate, the amount of which was measured by the method of Ya. Ph. Turakulov et al. [3]; that of phosphodiesterase by the method of R. I. Tatarskaya et al. [4], from the accumulation of p-nitrophenol split off from the potassium salt of di-p-nitrophenyl phosphate as substrate.

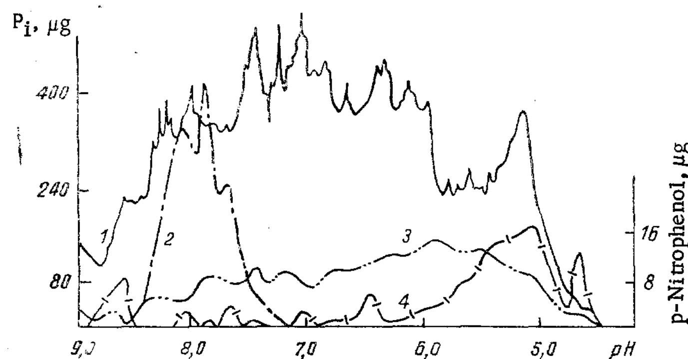

Fig. 1. Graph of the separation of Renard's viper venom (100 mg) with the aid of isoelectric focusing (time of focusing 20 h): 1) protein content (absorption at 280 nm); 2) 5'-nucleotidase activity (μg of P_i); 3) nonspecific alkaline monophosphatase activity (μg of P_i); 4) phosphodiesterase activity (μg of p-nitrophenol).

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In the process of separation, the 5'-nucleotidase was separated from the nonspecific alkaline monophosphatase and the phosphodiesterase and was focused in a narrow pH zone from 7.3 to 8.15. Phosphodiesterase activity was detected throughout the course of separation with a small peak at pH 5.2-6.3, and a low activity of the nonspecific alkaline monophosphatase was focused at pH 4.8-5.8 (Fig. 1).

After the end of isoelectric focusing, the fraction containing the 5'-nucleotidase was collected separately. Calculation of the activity in the fraction showed that the yield of 5'-nucleotidase was only 10% and, moreover, a phosphodiesterase was detected in the same fraction, its yield amounting to 1% of the total phosphodiesterase activity of the whole venom. Practically no nonspecific alkaline monophosphatase activity was detected in the 5'-nucleotidase fraction.

The low activity of the enzymes of the fractions can be explained by their inactivation at the isoelectric point on separation in an ampholine pH gradient. The separation of the phosphodiesterase over a wide pH range (from 4.0 to 9.0) is possibly connected with a molecular heterogeneity of this enzyme. The existence of numerous forms of phosphodiesterase in snake venoms has been deduced from the distribution of the phosphodiesterase into several fractions when snake venoms are separated with the aid of chromatography or other protein-fractionating methods [5]. The acidic properties of the nonspecific monophosphatase at pH 4.8-5.8 find confirmation in the results of an electrophoretic study of the venoms of various Central Asian snakes [6].

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KINETICS OF THE ENZYMATIC HYDROLYSIS OF PHOSPHATIDYLCHOLINE BY VARIOUS SNAKE VENOMS. II.

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Earlier [1] for the enzymatic hydrolysis of phosphatidylcholine (PC) we used as sources of phospholipase A_2 the venoms of the snakes *Naja oxiana* Eich (cobra), *Vipera lebetina* L. (kufi), *Echis carinatus* Schneir (saw-scaled viper), *Ancistrodon halus* (Pall) (mamushi) and *Vipera berus* (common adder). There is information according to which phospholipase A_2 acts both in an alkaline and in an acid medium, but a pH of from 7.0 to 10.0 is used most frequently [2-4]. The object of the present investigation was to study the kinetics of the hydrolysis of PC at a pH of from 7.0 to 11.0 by the venoms of the saw-scaled viper, kufi, mamushi, and common adder.

The hydrolysis of the PC was carried out with the aid of a TTA3 instrument (Autotitrator, Holland). For each reaction the cell was charged with 10 mg of PC dissolved in 10 ml of diethyl ether, 5 ml of Tris buffer, 1 ml of venom (the venom was dissolved in 0.1 M Tris buffer at pH 7.0, the concentration of venom being 0.15 mg/ml), 0.3 ml of 0.1 M $CaCl_2$, and 1 ml of Triton X-100. As the titrant we used 0.1 N KOH. In view of the fact that 70% hydrolysis took place even in the first few minutes [4], the time of reaction was

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