A PHOSPHODIESTERASE OF THE VENOM OF A CENTRAL-ASIAN COBRA

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Among the enzymes of snake venom, particular interest is presented by the phosphodiesterases, which catalyze the hydrolysis of nucleic acids to nucleotides, including the selective cleavage of the bond of the terminal mononucleotide with the remainder of the nucleic acid molecules [1, 2]. Aspects of the practical use of phosphodiesterases have been well-presented in Björk's review [2]. A particular requirement set for an enzyme preparation is the separation of the phosphodiesterase from the interfering 5'-nucleotidase [2]. In the present paper we give the results of a determination of the phosphodiesterase activity of the venom of a Central-Asian cobra and of the fractions obtained by the separation of this venom on sulfoethyl-Sephadex C-50 [3].

We determined the activity of the phosphodiesterase by the method of Tatarskaya et al. [4] by the hydrolysis of calcium di(p-nitrophenyl) phosphate and the activities of the ATP pyrophosphatase and of the 5'-nucleotidase by hydrolysis of the sodium salts of ATP and of 5'-AMP, respectively [5, 6]. To calculate the specific activity, the amounts of protein in the samples were measured by Lowry's method [7].

On chromatography on sulfoethyl-Sephadex C-50, the venom was separated into ten fractions: 5'-nucleotidase activity was detected in fractions VII and VIII, and ATP-pyrophosphatase activity between fractions VI and VII [5]. We found phosphodiesterase only in fraction VI, where it appeared together with cholinesterase. The "tail" of the phosphodiesterase extended into the beginning of fraction VII; however, because of its low activity, it was not taken into account. By chromatography we succeeded in raising the specific activity of the phosphodiesterase by a factor of 18.6, with a total loss of activity of 31.7% (yield 68.3%). Practically no 5'-nucleotidase was found in fraction VI, but mutual contamination at the junction of fractions VI and VII is not excluded. The quotient obtained by dividing the specific phosphodiesterase activity by the 5'-nucleotidase activity, which characterizes the ratio of the two enzymes is as follows: in the venom it is 0.28, and in the preparation obtained (fraction VI) it rises to infinity. S. K. Vasilenko [8] also obtained a phosphodiesterase preparation containing less than 1% of 5'-nucleotidase impurity by chromatographing cobra venom on sulfoethylcellulose.

On being passed through Sephadex G-25 gel, the enzyme preparation (fraction VI) separated into two components: the phosphodiesterase was separated from the cholinesterase and issued in a fraction which we have called VI-C-2, while the cholinesterase activity was bound to the first component. The bulk of the protein of the enzyme preparation was found in fraction VI-C-2. The specific activity of the phosphodiesterase had increased slightly (purification factor 19.2), but large losses of activity were found (to 68.3%). These losses, which are due to the partial inactivation of the enzyme in the process of its freeze-drying and Sephadex gel-filtration also reached a considerable magnitude (22%) according to Björk [2]. The phosphodiesterase obtained in fraction VI was accompanied by small amounts of ATP-ase [5]. After Sephadex gel-filtration the activity of the ATP-ase in fraction VI-C-2 was extremely slight, and its yield amounted to only 3.2%. The separation of the ATP-pyrophosphatase from the phosphodiesterase in the process of chromatography, the decrease in its specific activity, and the low yield in fractions VI and VI-C-2 as compared with that of phosphodiesterase permits the rejection – at least for the phosphodiesterase that we studied – of the suggestion that the ATP-pyrophosphatase action of venom is brought about by phosphodiesterase, which readily attacks both phosphodiester and acid anhydride bonds [9].

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