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Vipera a. ammodytes venom and its effect on the haemostatic system

Snake venoms are a rich source of proteins acting on the haemostatic system, *i.e.* platelet aggregation, coagulation cascade and fibrinolysis. Many of these components have already proved as useful for developing novel treatment agents for several vascular thromboembolic disorders.

For this reason we are systematically analysing *Vipera a. ammodytes* venom for new haemostatically active components. After gel-filtration of the whole venom, fractions were tested for different effects on the haemostatic system. It was shown that certain components of the *Vipera a. ammodytes* venom can degrade fibrinogen, dissolve fibrin clots *in vitro* and also activate blood coagulation factors X and II, as well as protein C. By using different inhibitors we tried to determine whether these effects are caused by metallo- or serine proteinases. In the future, effects on platelet aggregation, coagulation time and activation of plasminogen will be also tested. For systematic survey of the *Vipera a. ammodytes* venom components we optimized also a two dimensional gel electrophoretic analysis of the raw venom. 208 protein spots were detected and excised. Proteins were in gel trypsinized and resulting peptides analyzed with mass spectrometry. Until now, we identified a P-II and a P-III metalloproteinase (MP), an L-amino acid oxidase, three cysteine-rich secretory proteins (CRISPs), a nontoxic secretory phospholipase A_2 (ammodytin I_2) and a C-type lectin-like protein.

Ammodytase, a fibrin(ogen)olytic metalloproteinase, has been isolated from *Vipera a. ammodytes* venom in several chromatographic steps. Due to its activities, this enzyme is a potential thrombolytic agent to be used in medicine. In order to obtain sufficient amounts of ammodytase for further biochemical and pre-clinical characterization, a recombinant protein is needed. We amplified nucleotide sequences of MPs from a cDNA library of mRNA transcripts from *Vipera a. ammodytes* venom gland. Primers were constructed on the basis of nucleotide sequences common to known snake venom MPs. According to the length of the PCR products, they belong to all three classes of snake venom metalloproteinases (P-I to P-III). After cloning of the PCR products corresponding to the lenght of the P-II and P-III MPs, several nucleotide sequences were obtained, but the sequence of ammodytase was not among them. For this reason we continue determining the primary structure of ammodytase using MS analysis and the Edman sequencing. Until now, about 20% of the sequence has been revealed. On the basis of ammodytase's specific structure elements, primers will be designed, which will enable the identification of its cDNA and subsequent preparation of the recombinant protein.