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Note

Fast protein liquid chromatography of *Echis carinatus* venom

J. E. DYR*, H. FOŘTOVÁ and Z. VODRÁŽKA
Institute of Hematology and Blood Transfusion, Prague (Czechoslovakia)
and
F. KORNALÍK
Institute of Pathophysiology, Charles University, Prague (Czechoslovakia)
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The snake venom of the saw-scaled viper *Echis carinatus* has proved to be a valuable source of the procoagulant proteolytic enzyme ecarin[1–3]. Recently, it has been shown that this venom also contains several fibrinogenolytic enzymes[4] which may contribute to the reported defibrination effect of *Echis carinatus* envenomation *in vivo*[5,6]. These enzymes have not yet been fully separated and characterized, mainly owing to the lack of an appropriate method for the high resolution (with small available amounts) of the complex mixture of proteins that are present in the crude venom. This paper reports on the feasibility of fast protein liquid chromatrography (FPLC) of *Echis carinatus* venom using an anion-exchange column and a salt gradient.

EXPERIMENTAL

The lyophilized crude venom of *Echis carinatus* was dissolved in and dialysed against 0.02 *M* Tris buffer (pH 8.0). The samples were applied to a Mono Q prepacked HR5 column (Pharmacia FPLC system) and eluted with a linear sodium chloride gradient in the same buffer. The fractions belonging to the individual eluted peaks were pooled and assayed for their activity. Coagulation activity was tested according to Schieck *et al.*[1].

The rate of hydrolysis of chromogenic substrate designed for thrombin (T.S., Tos–Gly–Pro–Arg · pNa · HCl; Pentapharm, Switzerland) was measured spectrophotometrically at 405 nm[4]. The fibrinogenolytic activity was tested by means of sodium dodecylsulphate gel electrophoresis[4,7,8]. The degradation of fibrinogen was also determined from the prolonged clotting time of fibrinogen (Grade L, KABI, Stockholm, Sweden) incubated with the isolated venom fractions. The value of $(c_{F_i} - c_0)/c_0$ was taken as a measure of fibrinogen degradation c_{F_i} denotes the clotting time of fibrinogen, 100 μl, 3 mg/ml, incubated with the individual fraction F_i, 20 μl, for 10 min at 37°C after addition of 100 μl of thrombin, 2 NIH units/ml; c_0 is the clotting time of fibrinogen incubated with buffer instead of venom fraction).

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RESULTS AND DISCUSSION

The best FPLC result achieved under optimal conditions is shown in Fig. 1. A sample of 25 mg of *Echis carinatus* venom was fractionated into more than twenty peaks, which is a much more better resolution than had been obtained using DEAE-Sephacel chromatography[4] or polyacrylamide gel electrophoresis. The separation of the crude *Echis carinatus* venom using FPLC indicates that this venom contains a large number of so far undescribed proteolytic enzymes.

The procoagulant activity was separated into two peaks, which is in accordance with the previous observation[4] indicating that ecarin exists as at least two charge isomers. The fibrinogenolytic activity of both of the procoagulant fractions was very low and was completely inhibited by the reagents which have been shown to inhibit the procoagulant activity of ecarin[2,3] (2.6 m*M* EDTA, 10 m*M* cysteine). These results suggest that ecarin may possess both of these activities.

It is interesting that only one fraction (indicated in Fig. 1 by the bar) was capable of dissolving fibrin (formed from fibrinogen, 100 µl, 3 mg/ml, by simultaneous addition of thrombin, 100 µl, 2 NIH units/ml, and 20 µl of the individual venom fraction) during incubation for 24 h at 25°C. Moreover, the fibrin formed in the presence of the indicated fraction was completely dissolved after incubation for only 1 h. Further characterization of this fraction has shown that the enzyme responsible for the fibrinolytic activity is a single-chain protein of molecular weight 23,000.

The results demonstrate that FPLC is an efficient and useful method for the rapid and efficient separation of crude *Echis carinatus* venom, which is a complex mixture of proteins.

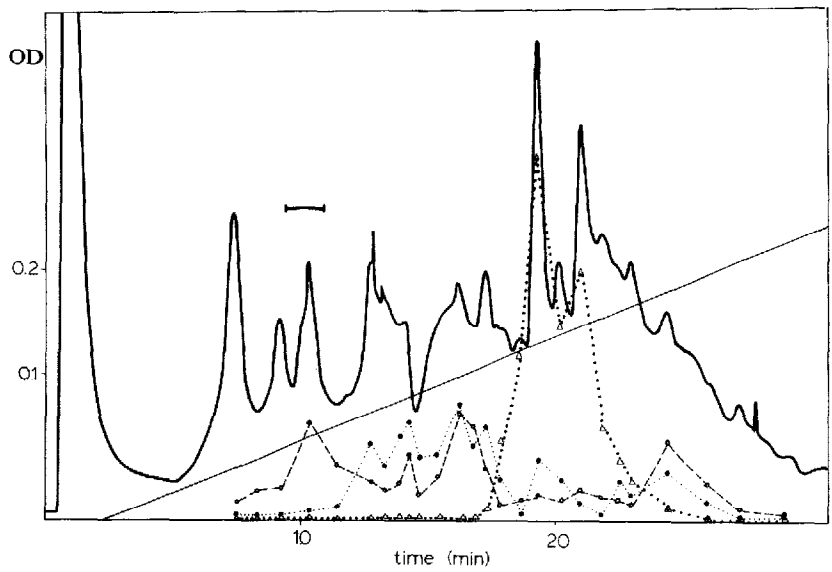

Fig. 1. Fast protein chromatography of crude *Echis carinatus* venom on Mono Q anion exchanger. Sample, 25 mg; flow-rate, 0.5 ml/min; eluent, 0.02 *M* Tris (pH 8.0) with an NaCl gradient up to 0.35 *M*. △ · · · △, Coagulation activity × 5 · 10^{-3} (units/ml); ● · · · ●, activity to T.S. × 10^2 (OD units/min); ○ – – ○, fibrinogenolytic activity, $(c_{F_i} - c_0)/c_0$.

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