ISOLATION AND CHARACTERIZATION OF KALLIKREIN – LIKE ENZYME FROM RUSSELL'S VIPER VENOM

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Introduction

Snake venom is a complex mixture of proteins with a variety of hydrolytic enzymes. For instance, the venom of viperidae family is particularly rich in Serine proteases, metalloproteases and various kinds of other polypeptides (Kisiel, 1979). These have a great impact on the role of incapacitating and immobilizing the digesting prey. These proteins have been modified to alter the functions of some physiological systems after the envenomation.

Russell's viper is considered as one of the deadly poisonous snakes in the world. Even in Sri Lanka, Russell's viper bites are responsible for the most number of deaths. Upon envenomation of Russell's viper venom, there are marked effects on circulatory system related with blood coagulation cascade in intrinsic pathways. Other symptoms such as tenderness and swelling at the bite area, blistering, systematic bleeding, kidney failure, hypotension, inflammation, myonecrosis, edema and pain are displayed.

In the past there had been a number of researches reported on isolation and characterization of various enzymes but little has been done on kallikerein –like enzymes (RVV-Ka) of viper venom (Bjarnason, 1983). Therefore in this report we have focused on the isolation and characterization of a kallikerein –like enzyme and its functional properties.

Materials and Methods

Crude venom was milked using healthy adult Russell's vipers reared in animal house, Faculty of Medicine, Peradeniya. Mono Q anion exchange column, FPLC were from Pharmacia. The gel filtration materials, Sephacryl S- 200, kallikerein specific substrate such as Benzoyloxycarbonyl (*BOC*) – Phe-Arg-MCA, Boc-Leu-Thr-Arg-MCA, Boc-Ile-Glu-Gly-Arg-MCA and Pro-Phe-Arg-MCA were from Wako pure chemicals Japan. PMSF and pepstatin were from Sigma chemicals. All other chemicals and reagents were from Wako pure chemicals, Japan and Sigma chemicals, USA.

Fractionation of RVV-Ka

FPLC Mono Q anion exchange chromatography was carried out in room temperature. 600 µl of crude venom were diluted with 600 µl of 20 mM Tris-HCl, pH 7.4 buffer. The column was eluted with a liner gradient of 0-0.5 M NaCl in 20 mM Tris-HCl, pH 7.4 buffer. One ml fractions were collected.

Isolation of RVV-Ka

RVV-Ka presenting fractions were isolated using Kallikrein specific MCA fluorogenic substrate. Florescence was observed at 460 nm emission and 380 nm excitation. Z-F-R-MCA and P-F-R MCA were used as substrates and were dissolved in 0.5 M Tris-HCl, pH 7.5.

Purification of RVV-Ka and determination of Molecular weight

Sephacryl S-200 column gel filtration was used to determine the molecular weight of RVV-Ka. Sephacryl S-200 column was equilibrated with 20 mM Tris-HCl buffer, pH 7.4. Three ml fractions were collected. Molecular weight of RVV-Ka was determined using standard proteins; cytochrom C (12,000 Da); Bovine Erythrocyte from carbonic anhydrases; 29,000 Da Bovine serum albumin ; 66,000 Da.

Characterization of RVV-Ka

Several proteinase inhibitors were used to determine the peptidase family.

Phenylmethanesulphonylfluoride (PMSF) Tosyl phenylalanyl chloromethyl-ketone (TPCK), Elastain, EDTA, Benzamidine, Diisopropylflurophosphate (DFP), P-Amidinomethanesulphonyl- fluoride (pAMSF), (100 µmol) were assayed with RVV-Ka Enzyme.

Optimum temperature was determined by assaying the mixture at 20^0 C, 25^0 C, 30^0 C, 35^0 C, 40^0 C, 45^0 C, 50^0 C, 60^0 C and 70^0 C. Thermal stability was determined at 20^0 C, 30^0 C, 40^0 C, 50^0 C and 60^0C. RVV-Ka was assayed in several buffer systems; 0.5 M Tris-HCl (pH 7.5 – 8.5), 0.5 M Sodium Borate (pH 8.0 – 11.0), 0.5 M phosphate buffer (pH 6.0 – 7.5) and 0.5 M Acetate buffer (pH5.0 – 6.0) to determine the optimum pH.

Result

Mono Q anion exchange column separated Russell's viper crude venom into five distingue peaks with significant absorbance at 280 nm. Among the collected fractions only fraction numbers 26 to 32 gave the high fluorescence values with MCA substrate of Z- FR-MCA and P-FR-MCA. Fluorescence values were highest with the fraction number 27. Molecular weight of the RVV-Ka was found 29 kDa by Sephacryl S- 200 gel filtration. Optimum temperature was 45^0 C and thermal stability was high at low temperatures, 20^0 C.

Effect of enzyme inhibitors

Significant inhibition of activity was observed only with serine protease inhibitors such as PMSF and p-AMSF Benzamidine.

Discussion

The overall mechanism of the toxic action of RVV-Ka is somewhat unclear in Russell's viper venom. But in general Russell's viper venom, mainly acts on blood clotting mechanism, which has serious implications for kidney function. Kallikerin acts on FXII (Hageman factor) in intrinsic blood oagulation pathway. The Mono Q fractionated sample showed activity only with Kallikrein specific substrate. It also showed the Arg-MCA cleavage security. This indicates a strong similarity with plasma Kallikrein which is the enzyme involved in releasing Bradykinin (BK) from high molecular weight kiniogen. The

molecular weight also coincides with the published details of kallikerin enzyme molecular weight.

The enzyme activity inhibition by the serine proteases inhibitors reveals that the enzyme belongs to the serine peptidases family.

References

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