Barnetobin, A new coagulant principle from the venom of Bothrops barnetti Peruvian snake.

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Bothrops barnetti is the most important venomous snake of northern Perú, inhabiting the coastal, Andean and jungle regions. A thrombin like enzyme, named barnetobin, was purified from de venom of this species by two steps of gel-filtration on Sephadex G-100, and ion-exchange chromatography on CM- Sephadex C-50. The enzyme was purified 52 fold with 31% of yield. Physicochemical studies indicated that the purified enzyme is a 52 kDa monomeric glycoprotein on SDS-PAGE under reducing conditions, which decreased to 27 kDa after deglycosylation with PNGase F. Barnetobin showed catalytic activity upon substrates, such as Fibrinogen, plasma, BApNA, TAME, BAEE and Chromozym TH and caused defibrinogenation when sub caudal vein administered to mouse. The proteinase split off fibrinopeptide A rapidly from bovine fibrinogen; however, only negligible traces of fibrinopeptide B were observed. The coagulant specific activity of the enzyme was equivalent to 251.7 NIH thrombin U/mg on bovine fibrinogen. The coagulant and amidase activities were inhibited by PMSF, soybean trypsin inhibitor and DTT. In contrast, β-mercaptoethanol, TLCK, EDTA and Heparin had little or no effect on its amidase activity. The complete cDNA sequence of Barnetobin with 750 bp encodes open reading frames of 233 amino acid residues, which conserve the common domains of thrombin-like serine proteases. Barnetobin shows homology with other thrombin like enzymes from snake venoms where common aminoacid residues were identified as those corresponding to the catalytic site and subsites S1, S2 and S3 already reported. In this study, we also demonstrated the importance of N-Linked glycans to improve thrombin-like activity of Barnetobin.

Keywords. Bothrops barnetti, thrombin like enzyme, glycosilation