
Conclusion

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In the present investigation, the biochemical and pharmacological properties of two non-toxic plasmin-like serine proteases, namely Bacethrombase and Brevithrombolase, were purified from indigenous rice-beer-starter-culture-borne *Bacillus cereus* strain FF01 and *Brevibacillus brevis* strain FF02B, respectively. This study was conducted to demonstrate the *in vivo* therapeutic application of these purified fibrin(ogen)olytic enzymes in dissolving the thrombin clots.

Both enzymes were purified by a combination of hydrophobic interaction and gel-filtration chromatography techniques to obtain homogenous enzymes. The molecular mass of Bacethrombase and Brevithrombolase was found to be 39.5 kDa and 56 kDa, respectively. The secondary structure of both enzymes showed similarity to the polyproline-II type model.

Amino acid compositions and PMF analysis clearly suggested that Bacethrombase and Brevithrombolase are new/previously uncharacterized fibrinolytic/fibrin(ogen)olytic serine proteases, that are plasmin-like enzymes. Furthermore, the *in vivo* thrombolytic efficacy of Bacethrombase and Brevithrombolase were found to be comparable to that of the commercial thrombolytic agent's plasmin and streptokinase. However, they did not display the *in vivo* toxicity, a major problem encountered with commercial thrombolytic drugs.

Till date, the understanding of the structure-function relationships in fibrin(ogen)olytic enzymes is contradictory and fragmentary data have been presented to explain their anticoagulant action. The present study attributes the anticoagulant activity of Bacethrombase to the enzymatic hydrolysis of fibrinogen. In contrast, the remarkable *in vitro* and *in vivo* anticoagulant potency of Brevithrombolase is attributed to its preferential degradation of thrombin, which is a unique mechanism to prolong blood coagulation.

The prolonged *in vitro* fibrinogen clotting and defibrinogenating activities of Bacethrombase contribute to its pharmacological importance for the treatment of hyperfibrinogenemia-associated disorders.

The lack of lethality and strong anticoagulant activities of Bacethrombase and Brevithrombolase in experimental animals, at a dose much higher than the therapeutic dose of commercial clot-bursting drugs, encourage their use in further clinical trials for the treatment of DIC.

Overall, the combined data indicate that Bacethrombase and Brevithrombolase are attractive alternatives for the treatment and/or prevention of cardiovascular disorders such as thrombosis. However, further bio-availability studies, using drug delivery systems that target thrombus, for developing more potent cardiovascular drugs are recommended.