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Anti-inflammatory activity of a serine protease produced from Bacillus pumilus SG2

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Highlights

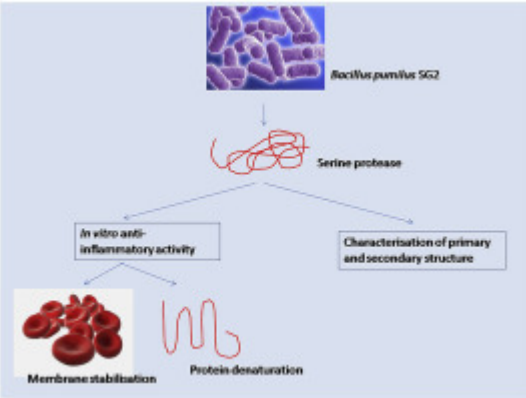
- This study investigated the anti-inflammatory activity of a protease isolated from Bacillus pumilus SG2 and also analysed it's 1° and 2° structure.
- Anti-inflammatory potential of SG2 protease was assessed using in-vitro models.
- The SG2 protease was found to possess anti-inflammatory potential much comparable to the standard drug, diclofenac.

- The 1° and 2° structure characterisation of the enzyme will help in enzyme engineering.

Abstract

Proteases have appreciable anti-inflammatory activity and proteolytic enzymes from diverse sources have been studied for their anti-inflammatory potential. This study investigated the anti-inflammatory activity of a protease isolated from Bacillus pumilus SG2 using in-vitro models such as heat and hypotonicity induced hemolysis and protein denaturation. The activity exhibited by SG2 protease was comparable to that exhibited by the standard drug diclofenac. The IC 50 value of SG2 protease for inhibition of heat induced hemolysis was calculated to be 226 μg while that of diclofenac was 215 μg The IC 50 value for both protease and diclofenac for the inhibition of hypotonicity induced hemolysis was 85 μg. The IC 50 value of SG2 protease for the inhibition of protein denaturation was 247 μg while that of diclofenac was 181 μg. The structure of SG2 protease was deduced using online tools. The enzyme had a signal peptide of 31 amino acids and a pro-peptide of 77 amino acids. The mature protein consisted of 298 amino acids. The catalytic triad, oxyanion hole and secondary structure of SG2 protease was also studied. Thus a protease with anti-inflammatory potential was studied and was structurally characterized, the details of which may help in engineering the enzyme.

Graphical abstract



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Introduction

Proteases are hydrolytic enzymes ubiquitous in nature with both physiological and commercial significance. Proteolytic enzymes have medicinal uses and several plant and microbial proteases have been isolated and their medicinal value has been evaluated. Proteases have developed as effective therapeutic agents (Kim et al., 2006).

Oral administration of proteases from *Aspergillus oryzae* (Luizym and Nortase) has been used as a digestive aid to correct certain lytic enzyme deficiency syndromes (Mikawlrawng, 2016). Clostridial collagenase or subtilisin is used in combination with broad-spectrum antibiotics in the treatment of burns and wounds (Riley and Herman, 2005). Proteases which can catalyse fibrinolysis have been reported (Kim et al., 2006). Proteases, both plant and microbial, have anti-inflammatory potential. Proteolytic enzymes are effective denture and contact lens cleansers. These enzymes are also used to treat necrosis, cancer and cardiovascular disorders (Hellgren et al., 1986; Chanalia et al., 2011).

Proteases are reportedly potential anti-inflammatory drugs. They have been proved to act independently or synergistically with non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are commonly used to treat inflammation. Nevertheless, the side effects of the use of NSAIDs are adverse and hence use of bioactives and bioenzymes with anti-inflammatory activity will help in decreasing the usage of NSAIDs (Swamy and Patil, 2008, Chanalia et al., 2011). Microbes are preferred to plants and animals as sources of proteases because they are generally cheaper to produce, their enzyme contents are more predictable and controllable, reliable supplies of raw material of constant composition are more easily arranged, and plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds, endogenous enzyme inhibitors etc (www.lsbu.ac.uk/biology/enztech/sources ↗). Microbes have undermined plants and animals as sources of enzymes due to their broad biochemical diversity, ease of mass culture and also to the ease with which they can be genetically modified (Ishwarya and Sangeetha, 2013).

We had earlier reported the production and purification of a protease from *Bacillus pumilus* (Sangeetha et al., 2010). In the present study we have analysed the secondary structure of protease and investigated the anti-inflammatory potential of *Bacillus pumilus* protease using in vitro models.

Section snippets

Enzyme production

A promising strain *Bacillus pumilus* SG2 which produced protease was isolated and maintained on agar slants at 4 °C. The production medium consisted of (w/v) 0.04% CaCl2, 0.02% MgCl2, 1% glucose, 0.5% NaCl and 0.3% yeast extract (in sodium phosphate buffer, pH 9.0). Five ml of overnight culture (O.D600=1.0) of *Bacillus pumilus* SG2 was inoculated into 100 ml production medium and incubated on a rotary shaker (180 rpm) for 48 h at 37°C. At the end of the incubation period, the production medium

Results and Discussion

Inflammation is a reaction process invoked by several physical and chemical agents, infections and diseases. The process of inflammation is manifested as heat, redness, edema and pain, all of these caused primarily by the damage to tissue proteins and release of lysosomal enzymes. Hence, inhibition of protein denaturation and stabilisation of lysosomal membranes may prevent the onset of inflammation. The efficacy to inhibit protein denaturation and membrane lysis will apparently indicate

Conclusion

A protease was produced from *Bacillus pumilus* SG2 and was studied for its anti-inflammatory potential. The enzyme was able to inhibit heat and hypotonicity induced hemolysis and protein denaturation. The efficacy to inhibit hemolysis and denaturation proved that the enzyme has anti-inflammatory potential and the IC 50 values observed with the inhibition studies implied that the enzyme SG2 protease is as potent as the standard drug diclofenac. The secondary structure of SG2 protease was also

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