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Cloning and characterization of serpin from red king crab *Paralithodes camtschaticus*

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Abstract

Serpins are a family of serine protease inhibitors that are involved in numerous physiological processes and are known to regulate innate immunity pathways. To advance our understanding of their role in *P. camtschaticus*, a commercially significant species, we cloned and characterized a serpin from this species, designated serpin PC, that has anticoagulant and anticomplement effects on human blood. We found that serpin PC is a secreted protein with a typical serpin-like primary structure that is similar to other known crustacean serpins. Recombinant serpin PC was found to have inhibitory activity against R/K-specific bovine cationic trypsin. The reaction proceeds through the formation of a stable covalent complex of peptidase with P1 residue R383 of serpin PC. This interaction is characterized by a relatively high overall inhibition constant $k_{ass}=(2.3 \pm 0.7)\times10^6$ $M^{-1}s^{-1}$ and an SI of 4.7 ± 0.8. Protein localization by western blotting showed that serpin PC is present in the muscles and, to a lesser extent, the heart, whereas it is transcribed predominantly in hemocytes and the heart. Through peptidase activity profiling of hemocytes and plasma, we found that serpin PC inhibits at least two R/K-specific activities and showed that it inhibits phenoloxidase (PO) activity induction in hemocytes.

Highlights

- A serpin PC from red king crab, *P. camtschaticus* was cloned and characterized
- Though transcribed in hemocytes and the heart, the protein was found in the muscles and heart
- Serpin PC inhibits at least two R/K-specific peptidases in hemocytes and plasma
- In hemocytes, serpin PC suppresses PO activity induction

Keywords:

serpin, crab, Paralithodes camtschaticus, immunity, phenoloxidase, inhibitor, recombinant, refolding